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USE OF COPPER SULFATE TO CONTROL *HAEMONCHUS CONTORTUS* INFESTATION IN HAMPSHIRE EWES

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ABSTRACT OF THESIS

USE OF COPPER SULFATE TO CONTROL *HAEMONCHUS CONTORTUS* INFESTATION IN HAMPSHIRE EWES

Two studies were conducted to evaluate the effectiveness of using copper sulfate ($CuSO_4$) as a drench in Hampshire ewes to control stomach worms (*Haemonchus contortus)*.

A study was conducted to assess the effectiveness of $CuSO_4$ to control gastrointestinal nematodes (GIN) over a three year period. Ewes were FAMACHA scored, hematocrit evaluated for packed cell volume (PCV), and fecal egg counts (FEC) were determined from 2007 through 2009. Ewes received only $CuSO_4$ to control GIN. Ewes with FEC exceeding 6,000 eggs/g feces were drenched.

A separate study during the summer of 2008 assessed the potential of $CuSO_4$ drench to cause copper toxicity in Hampshire ewes. Eighty-four ewes were blocked to one of two treatments according to parity and balanced for FEC. One group received $CuSO_4$ (D) and the other was not drenched (ND). Jugular blood samples were collected at pre-determined intervals after $CuSO_4$ was administered to D ewes. Serum was analyzed for aspartate aminotransferase (AST), alanine aminotransferase (ALT), and creatine kinase (CK). Elevated serum levels indicate copper toxicity.

Results suggest $CuSO_4$ has the potential to control stomach worms in Hampshire ewes without causing copper toxicity.

KEYWORDS: Copper Sulfate, Copper Toxicity, *Haemonchus contortus*, Hampshire Ewes, Weaning

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July 25, 2011
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THESIS

Melinda Mallory Simpson

The Graduate School

University of Kentucky

2011

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THESIS

A thesis submitted in partial fulfillment of the
requirements for the degree of Master of Science in the
College of Agriculture
at the University of Kentucky

By

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Lexington, Kentucky

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Lexington, Kentucky

2011

To My Dad, I know you would be proud.

And to baby Jacob, for providing extra motivation.

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the safety of consumers. Thank you for serving on my committee and for providing feedback on my thesis.

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the loss of numerous loved ones and my mom has always been, and continues to be, the foundation that my brother, sister and I rely on

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER I

Introduction

The Hampshire is one of the most popular sheep breeds in the United States. As one of the larger breeds, the Hampshire is acknowledged as a specialized sire breed where the rams are used as a terminal sire in the production of heavy weight slaughter lambs. Characteristics of the breed include moderate prolificacy, average milking ability, excellent growth and carcass cutability, and the ability to produce a medium-wool fleece.

In the United States, rams are typically turned out with ewes in the middle of August and are removed in the middle of October to enable Hampshire ewes to lamb in January and February. Lambs are weaned at 60 days of age in March and April. In the south, these particular months are prone to warming temperatures and increasing rainfall when compared to previous winter months. Ewes and lambs maintained on pasture are at higher risk of internal parasite infestation, specifically the stomach worm *(Haemonchus contortus)*. The lactating ewe can harbor hypobiotic stomach worms from previous infestations and the increasing temperatures and rainfall trigger development to resume and consequently re-infest herself, other ewes, and lambs. This particular parasite is responsible for significant production losses for sheep producers each year.

Sheep producers use commercial anthelmintics to control stomach worm infestations. Overuse of these products has led to the development of anthelmintic resistance. As a result, producers are in search of alternative techniques to monitor and control parasites. These include the FAMACHA system and Fecal Egg Counts (FEC). An alternative to commercial anthelmintics is copper sulfate ($CuSO_4$), a popular method of worm control in the 1950's and before commercial anthelmintics became available. Now that resistance to commercial anthelmintics is commonplace, $CuSO_4$ may again be a viable option for stomach worm control in sheep.

CHAPTER II

Literature Review

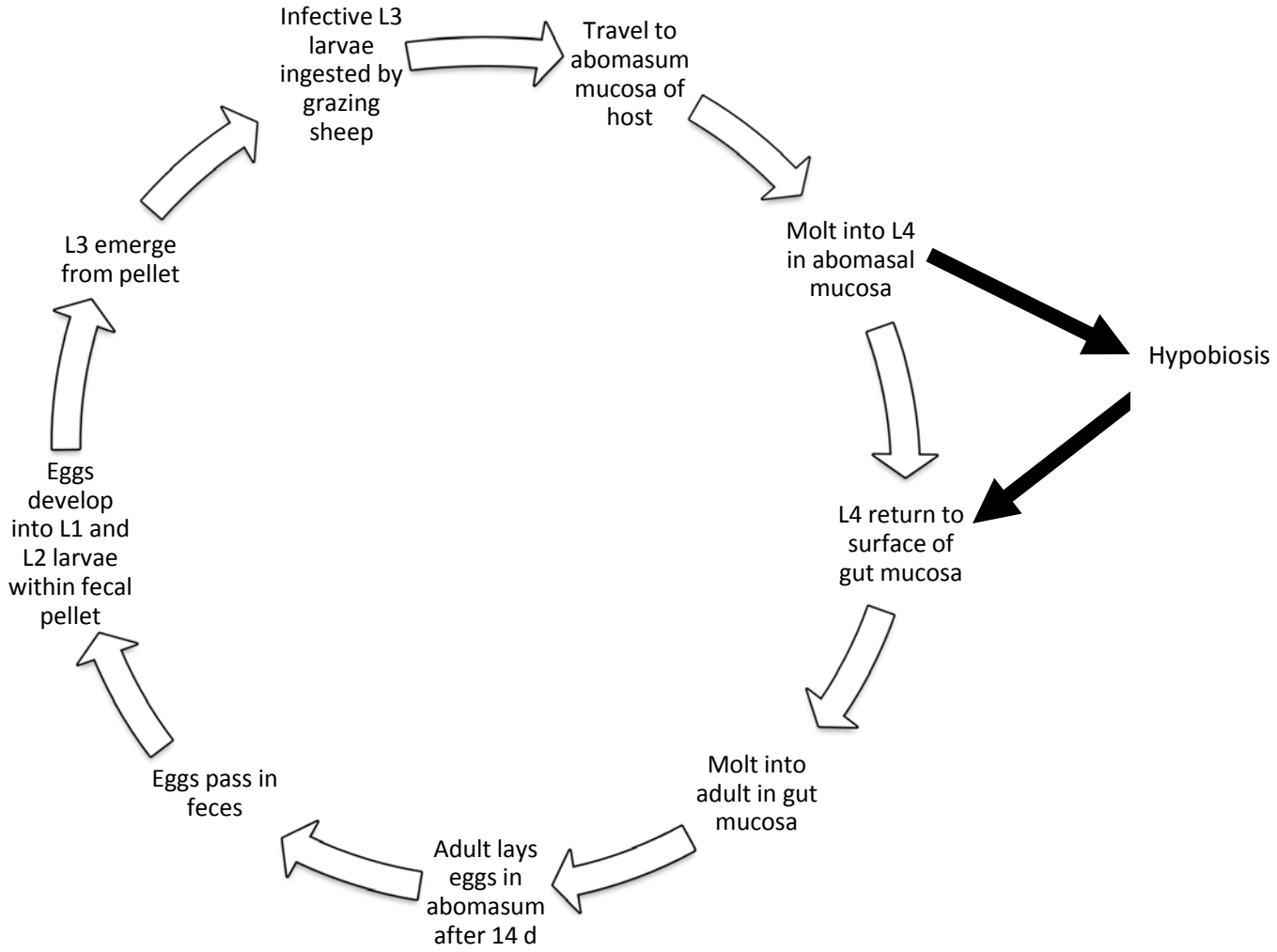
Haemonchus contortus

Gastrointestinal nematodes (GIN), *Haemonchus contortus* (Barber Pole Worm) in particular, have plagued small ruminant producers worldwide for many years. The southeastern United States and other areas, with hot and humid summers, present the most favorable environmental conditions for *H. contortus* to thrive. Although the financial impact of these nematodes is difficult to quantify, losses to producers occur through decreased production, cost of prevention, cost of treatment, and the deaths of infected animals. In order to appreciate the complexity of these parasites, we must have knowledge of their lifecycle, be able to recognize infestations, and, ultimately, know how to control them.

Lifecycle. In order to complete their 21-d lifecycle (Figure 2.1), the *H. contortus* parasite must develop and lay eggs in the host. Sheep and other small ruminants ingest the infective L3 larvae when grazing. Once ingested, these larvae invade the abomasum where they take one of two paths. They can either molt (structural and physiological changes) into L4 larvae after shedding their sheath and, eventually, become adults or they can enter a state of dormancy referred to as hypobiosis (Hempworth et. al., 2006). Molting allows the parasites to cope, once inside the host, and aids parasite entry into a new environment (Gibbs, 1982). Larvae (L4) enter hypobiosis under unfavorable environmental conditions, such as hot and dry or freezing weather, and remain metabolically inactive without threat to the host until a signal is received to continue development. Although the details of the signaling are not completely understood, it is theorized to involve immune and environmental cues (Miller and Horohov, 2006). Gibbs (1982) describes hypobiosis as one of the most useful lifecycle adaptations to ensure parasite survival. Not only does hypobiosis ensure survival of parasites during periods of environmental adversity, it is useful in synchronizing the lifecycle to changing environmental or host conditions.

Upon molting into L4 larvae, the parasite undergoes a final molt in the abomasum

Figure 2.1. Lifecycle of *Haemonchus contortus.*[1]



[1]Adapted from Hempworth et. al. (2006) and incorporating information from Miller and Horohov (2006).

to become an adult. Females begin laying eggs within 14 d after the final molt and can lay up to 5,000 /d (Hepworth et. al., 2006). These eggs exit the body in the feces and, if favorable environmental conditions are present, hatch and develop into L1 and L2 larvae within the excreted fecal pellet. The infective L3 larvae emerge from the pellet and migrate up the blades of grass to be consumed by the grazing animal (Hempworth et. al., 2006).

Parasitic Survival. Numerous studies have been conducted to evaluate the survival of *H. contortus* under a variety of environmental conditions. It has been demonstrated that eggs of this nematode in fresh sheep feces are affected by drying, lack of oxygen, heat, and cold (Shorb, 1944; Dinaburg, 1944). Additionally, researchers at the University of Kentucky established the ability of *H. contortus* to survive on pasture over the months of October through April (Todd et. al., 1949).

One characteristic of the *H. contortus* that makes it difficult to manage is the hardiness of the L3 larvae. After the L3 development stage is complete, larvae are less susceptible to unfavorable environmental conditions. Hardiness has been attributed to the L3's ability to migrate to a more favorable microenvironment and development of the larval sheath, which provides protection from desiccation (O'Connor et. al., 2006). Ellenby (1968) conducted experiments on ensheathed (with sheath) and exsheathed (without sheath) larvae kept in a 47% relative humidity chamber maintained at approximately 18°C. Slides containing the specimens were removed at different intervals and placed in moist chambers where the revival rate of the larvae was examined until it reached its maximum. Ellenby (1968) concluded that ensheathed larvae of *H. contortus* survived desiccation more successfully than exsheathed larvae. After 1 h exposure to 47% relative humidity, the survival of exsheathed larvae was described by Ellenby as poor. After 8 h, no larvae were recovered. Ensheathed larvae exposed to the same relative humidity were all recovered after 30 h of exposure and some were even recovered after 3 weeks. With regard to water loss, fully hydrated living larvae, both ensheathed and exsheathed, have a water content of 75%. Exsheathed larvae exposed to 47% relative humidity had a water content of 10% after an hour of exposure (Ellenby, 1968). Ensheathed larvae will also dry very rapidly by loosing half their water content

within the first 15 min. of drying However, water loss then begins to stabilize, and after 24 h, water content was 30% (Ellenby, 1968). This work showed the sheath dried first when larvae were exposed to 47% relative humidity, which slowed the rate of drying, thereby enabling a greater survival rate among ensheathed larvae.

There are three major GIN species in sheep, including *H. contortus*. The *Teladorsagia circumcincta* and *Trichostrongylus colubriformis* are dominant parasites in winter months and areas with uniform rainfall. Compared with *H. contortus*, the *Teladorsagia* and *Trichostrongylus* spp., including *T. colubriformis*, have a greater resistance to desiccation and have the ability to develop at lower temperatures (O'Connor et. al., 2006). Jasmer et. al (1986) examined the differences in survival of both eggs and larval mutations of eastern Washington isolates of *H. contortus* and *Ostertagia circumcincta*, a trychostrongylid species, when exposed to cold temperatures. Developing eggs were incubated at 10°C in feces for 48 h (*O. circumcincta)* or 96 h (*H. contortus)* before exposing both to -18°C. Incubation times were based on the approximate time required for eggs to become larvated in 10°C cultures. They found both species developed at 10°C. However, 95% of *O. circumcincta* and only 32% of the *H. contortus* eggs were larvated after 120 h. Egg survival was described as the ability of eggs to hatch after exposure to -18°C for 15 h (Jasmer et. al., 1986). Only 4% of *H. contortus* eggs in feces hatched under these conditions whereas 87% of *O. circumcincta* eggs hatched (Jasmer et. al., 1986). These results indicate the two trichostrongylid nematodes differ in their ability to tolerate cold temperatures with *O. circumcincta* being the most tolerant of colder temperatures. Therefore *O. circumcincta* would be more capable of overwintering in colder climates. Nevertheless, the *H. contortus* is capable of overwintering in the milder winters found in the southeast United States.

Each of the three GIN species is less susceptible to unfavorable climatic conditions after eggs have completely developed as L3 larvae (O'Connor et. al., 2006). However, moisture affects both movement and motility of larvae. The ability of the infective L3 larvae to survive longer in suboptimal environments than pre-infective, L1 or L2 stages, is theorized to be due to their ability to migrate to a more desirable microenvironment (O'Connor et. al., 2006). As the fecal pellets dry, developing larvae may desiccate and die (Stromberg, 1997). Stromberg (1997) also stated if the

environment is dry, surviving larvae movement onto surrounding herbage may not be possible. Instead the larvae may migrate into the soil beneath the fecal pat where they remain until the climate becomes more favorable to them. If ingestion by the grazing animal is the only way to complete the *H. contortus* lifecycle, obviously the environment plays a major role in this completion.

Susceptible Animals. All sheep have some nematodes in the abomasum (Miller and Horohov, 2006). Within a flock, the greater part of the nematode population is contained in a small portion of ewes (Torres-Acosta and Hoste, 2008). Kaplan et. al. (2004) estimated 20 to 30% of sheep within a flock harbor 70 to 80% of the worms. Severity of parasitic infections can be influenced by the nutritional status of the hosts (Knox et. al., 2003). To a point, well-fed animals are more apt to tolerate infestation than those receiving inadequate nutrition. At some point, the host's ability to function optimally is overcome by the infection. Miller and Horohov (2006) explained how nutrients are partitioned or directed to where they are most needed depending on the host's age, sex, season of the year, and exposure to parasitic or otherwise infectious agents. Nutrients are partitioned differently for growth, breeding, pregnancy, lactation, and immunity. Maintaining a correct balance, when partitioning nutrients, is essential to the well being of the host. An example of how parasites can influence nutrient partitioning involves damage to the gut mucosa associated with increased numbers of GIN. Damage to the gut mucosa reduces nutrient absorption, which forces the host to utilize stored body reserves. Ultimately the animal is weakened, which results in a loss of productivity (Miller and Horohov, 2006). More specifically, in the gastrointestinal tract, GIN cause an increased loss of endogenous protein partially due to plasma protein leakage and increased exfoliation of the epithelial cells of the gut and mucoprotein secretions (Poppi et. al., 1986; Bown et. al., 1991). Overall, the animal will have a net movement of protein from production processes including meat, bone, milk, and wool production and instead synthesize plasma proteins to repair the gastrointestinal tract and mucus secretions (Steel et. al., 1982; Symons, 1985; Bown et. al., 1986).

Lactating females are a specific group of animals targeted by GIN. Crofton (1958) found increased fecal egg counts in lactating ewes and then proposed the term

"periparturient" rise. He attributed the "periparturient" rise to hormonal changes associated with lactation. The "rise" suppresses immunity because of the blood consuming abilities of *H. contortus*. Gibbs (1982) and Courtney et. al. (1984) found a dramatic increase in fecal egg output 2 weeks before and up to 8 weeks after lambing. The post parturition rise in lactating ewes occurs when the immune system is compromised by the demands of pregnancy and lambing and can be further intensified by a suboptimal feed intake (Vlassoff et. al., 2001). This allows any larvae ingested over the lambing period to establish and begin to mature into adults. Leathwick et. al. (1995) proposed ingested larvae establishment (maturation) increased from 1 to 30% three weeks prior to lambing. Years later, Leathwick et. al. (1999) dosed lactating ewes with 12,000 L3 larvae of both *T. colubriformis* and *O. circumcincta* at either 2, 4, or 6 weeks after lambing. Twenty-five days later ewes were drenched and 3 d later sacrificed for worm counts. Nematode maturation was highest for *O. circumcincta* 2 weeks after parturition with a mean establishment rate of 6.1%. At 4 and 6 weeks post lambing, nematode establishment never exceeded 2%. Based on these results, these researchers determined that lactating ewes in New Zealand expressed the ability to prevent establishment of ingested larvae (Leatherwick, et. al., 1999). Therefore, ewes drenched while lactating should have the ability to overcome parasitic infection.

Signs of Infestation. Recognition of signs of internal parasite infestation is essential for control. Clinical signs of animals suffering from internal parasites include loss of appetite, weight loss, and weakness. According to Hempworth et. al. (2006), the *H. contortus* parasite is capable of consuming up to one-tenth of an animal's total blood volume in one day. Therefore, one of the primary indicators of a *H. contortus* infestation is anemia. Anemia can be identified by the color of the mucous membranes of the lower eyelid using the FAMACHA system (Figure 2.2). Uninfected animals have a red mucous membrane whereas anemic animals have light pink to white membranes (Hempworth et. al., 2006). The other primary indicator of *H. contortus* infestation is edema or "bottle jaw" (Figure 2.3). This condition is described as an accumulation of fluid under the lower jaw resulting from blood protein loss due to *H. contortus* infestation (SID, 2002).

Figure 2.2. FAMACHA anemia guide used to assess color of the mucosal membrane of the eye.[1]

[1]Photo courtesy of Melanie Hoar, University of Kentucky (2009).

Figure 2.3. Ewe exhibiting edema or "bottle jaw".[1]

[1]Photo courtesy of Dr. Debra Aaron, University of Kentucky (2008).

Techniques for Measuring Infestation. In the absence of newly developed commercial anthelmintics to control GIN in sheep, nematode resistance to anthelmintics was becoming a major concern 13 years ago (Waller, 1997). This spawned development of new techniques of parasite control. These new techniques are based on the principle of deworming only a minimal number of animals and, therefore, exposing only a minimal population of GIN to anthelmintics (dewormers). Deworming (drenching) only the portion of the flock suffering from significant GIN infestation leaves several animals untreated. Theoretically, untreated animals excrete worms that are not resistant to anthelmintics. Propagation of these worms can decrease exposure to anthelmintics and ultimately decrease the development of anthelmintic resistant worms.

One technique developed to evaluate the extent of *H. contortus* infestation is the FAMACHA system (Figure 2.2). This system was developed by Van Wyk and Bath (2002) and was based on the principle of treating only those animals unable to withstand a *H. contortus* challenge while maintained on pasture. This system estimates extent of clinical anemia to determine which animals to treat. In developing the system, sheep were classified into one of the following categories: red, red-pink, pink, pink-white, or white corresponding with 1, 2, 3, 4, or 5, respectively. Initial trials testing the FAMACHA system were conducted with 388 sheep on a South African farm located in an area prone to *H. contortus* infestation. All sheep were initially treated in March with levamisole for internal parasites before anthelmintic use was suspended for 125 d. Sheep were examined weekly for color of the conjunctival mucous membranes and submandibular edema or “bottle jaw”. Jugular blood samples were obtained from sheep categorized pink-white (4) or white (5) for microhematocrit (Ht) determination. Of the ewes with FAMACHA scores of 4 or 5, only those with Ht values at or below 15% were treated with anthelmintics. Color of the conjunctival mucous membrane was evaluated and Ht determinations were made on all ewes six times during the 125-d period. At the conclusion of the experiment, researchers determined drenching was reduced by an estimated 90% when considering that sheep were previously drenched at 3-week intervals during the *H. contortus* season. Seventy percent of sheep were not drenched and only 10% of sheep required more than one treatment. Assessment of anemia can be found in Table 2.1. Accuracy of FAMACHA scores were evaluated using Ht scores. Overall,

Table 2.1. Accuracy of anemia estimates based on FAMACHA scores during initial trials[a]

Estimate accuracy	Percentage of 2,367 observations
1. Correct category estimated	55.6
2. FAMACHA score estimated too low (more serious)	
a. Category 2 classed as 1	25.1
b. Category 3 classed as 1	8.4
c. Category 5 classed as 4	3.2
d. Category 4 classed as 1	2.0 [b]
e. Category 5 classed as 2	0.2 [b]
f. Category 4 classed as 2	0.2 [b]
g. Category 5 classed as 3	0.2 [b]
Total % too high	39.3
3. FAMACHA score estimated too high (less serious)	
a. Estimate 1 category too low	3.3
b. Estimate 2 categories too low	1.8
Total % too low	5.1

[a] Adapted from Malan et. al. (2001).

[b]Instances where incorrect classification was regarded to be potentially life threatening to the sheep.

anemia was correctly categorized in 55.6% of the total 2,367 estimates. Of the total estimates, 39.3% were estimated too high, and 5.1% were estimated too low. An important observation was when FAMACHA was not accurate, the majority of the time it was off by an adjacent category, i.e. a FAMACHA of 3 was estimated and the actual score was 2. Additionally, incorrect classification of anemia was only considered life threatening in 2.6% of the 2,367 total observations and these animals required salvage drenching in order to overcome GIN infestation.

The FAMACHA system was further developed through a series of experiments conducted on more than 30 commercial sheep farms representing a wide range of farming and management systems as well as various breeds in South Africa. Breeds were primarily Merino and Döhne Merinos, but also included Mutton Merinos, Dorpers, Ile de France, and Suffolks. Van Wyk and Bath (2002) provided a summary of these experiments. Within these trials, researchers were able to standardize the previous five informal descriptive categories into specific Ht ranges (Table 2.2) and develop a colored card with illustrations showing the conjunctival mucous membranes of sheep in the five Ht ranges (Figure 2.2). Bath and Van Wyk (2001) concluded at the end of the commercial trials that the FAMACHA system had been properly field tested and was ready to be released to farmers. Since its development, the FAMACHA system has proven to decrease the frequency and, therefore, cost of anthelmintic treatment (Malan et. al., 2001; Bath and Van Wyk, 2001; Molento et. al., 2009; Kaplan et. al., 2004).

Prevention. Management techniques used to prevent internal parasite infestations include mixed species grazing, pasture rotation, culling ewes with high FEC, and use of alternative forages. Barger (1999) stated that alternating among animal species (cattle, sheep, goats) that do not share common parasites can be a successful technique to promote worm control. Additionally, each host species can be used to prepare clean pastures for the other, thereby, increasing the interval required between anthelmintic treatments (Barger, 1999). Niezen et. al. (1996) demonstrated, on two organic farms in New Zealand, the ability to achieve acceptable parasite control in sheep by alternatively grazing with cattle. Rotational grazing is classified by Barger (1997) as an evasive strategy to control GIN, which includes a management system that relies on

Table 2.2. Relationship between FAMACHA score and hematocrit values[1]

	FAMACHA Score				
	1	**2**	**3**	**4**	**5**
Color	Bright red	Red-pink	pink	Pink-white	White
% Hematocrit value[2]	≥ 28	25 (23-27)	20 (18-22)	15 (17-13)	≤ 12
Hematocrit classification	Optimum	Acceptable	Borderline	Low	Fatal

[1]Adapted from Van Wyk and Bath, (2002).

[2]Parentheses represent corresponding ranges.

movement of livestock to another pasture before the number of larvae can reach a significant level. Michel (1976) recommended moving animals treated with anthelmintics to a safe pasture before the level of larvae in the original pasture could reach dangerously high concentrations based on seasonal peaks in the parasitic lifecycle. However, recent work by Torres-Acosta and Hoste (2008) disagree with the "dose and move" recommendation of Michel (1976) as this technique selects heavily for anthelmintic resistance (AR). Instead, they recommend to move and then dose with anthelmintics in order to protect the external portion (egg, L1, L2 and L3 larvae before ingestion) of the lifecycle that has not been exposed to anthelmintics (refugia). This technique endeavors to protect against AR.

Condensed tannins (CT) exhibit the potential to control internal parasites (Aerts et. al., 1999). Moderate levels of dietary CT (20 to 40 g of CT/kg diet DM) will hydrogen bond to protein at a near neutral pH (6.0 to 7.0) in the rumen to form CT-protein complexes (Barry et. al., 2001). These CT-protein complexes dissociate and release bound protein when the pH is less than 3.5 in the abomasum (Barry et. al., 2001). Therefore, plants containing CT are able to protect dietary protein from degradation in the rumen, which can increase the amino acid supply to both the abomasum and small intestine resulting in an improved nutritional status of the animal (Min and Hart, 2003). As previously stated, the nutritional status of the host does influence parasitic infection (Knox et. al., 2003). For this reason, CT may have an indirect effect on GIN by increasing the resistance and resilience of the animal through improved protein nutrition. Evidence of this can be seen in the work of Niezen et. al. (1998). Fecal egg counts and live weight gain of 160 Romney ewe lambs were compared when grazing six forage types (Niezen et. al., 1998). Three forages containing CT (Grasslands Goldie, Grasslands Maku, and Sulla) and three forages without CT (lucerne, plantain, and ryegrass/white clover) were used in this study. Twenty-five lambs were divided into two groups within each type of forage; 10 non-parasitized (NP) and 15 parasitized (P). The P lambs received no anthelmintics while NP lambs acted as non-parasitized controls because they were given anthelmintics (a combination of benzimidazole/levamisole) at 14-d intervals over a 42-d period. Fecal egg counts were performed on samples of all lambs prior to the start of the experiment. Lambs were randomly allotted to treatments based on fecal egg

counts and initial live weight. Ten lambs were slaughtered to provide parasite data before the trial began. Lambs were weighed and fecal sampled at 7-d intervals for live weight and FEC determinations. Live weight gains of P lambs grazing all forages were reduced, however, lambs grazing Grasslands Maku and Sulla gained more than those grazing Grasslands Goldie, lucerne, ryegrass/white clover, or plaintain. The only group with a significant reduction in FEC were lambs grazing Sulla. The authors acknowledged the need for further studies in this area to determine the forages to use in grazing systems to maximize lamb performance while decreasing anthelmintic use.

Commercial Anthelmintic Treatment. Treating animals with anthelmintics has provided producers with a simple, effective way to reduce the losses caused by GIN infections (Torres-Acosta and Hoste, 2008). However, overuse of commercial (industry developed) anthelmintics alone or in combination with poor management techniques, has encouraged the development of AR within the flock. Currently, many producers find themselves facing this problem. The following section will discuss AR in detail.

Anthelmintic Resistance. Waller (1997) describes AR as a state of industry crisis in some livestock sectors, namely small ruminants. Significant differences between and within the livestock industry can be found with regard to AR. For example, in regions with higher rainfall, widespread multispecies resistance has been recognized for a considerable time. Unfortunately, the more extensively worms are controlled with anthelmintics, the more likely resistance is to develop. Finding a lack of a response to anthelmintic treatment in parasitized animals is usually the first sign of AR nematodes on a farm (Torres-Acosta and Hoste, 2008). The most common management flaws associated with the development of AR include the overuse of anthelmintics (Waller, 1993; Jabbar et. al., 2006; Papadopoulos, 2008; Torres-Acosta and Hoste, 2008), underdosing (Waller, 1993; Jabbar et. al., 2006; Papadopoulos, 2008; Torres-Acosta and Hoste, 2008), using faulty equipment (Waller, 1993), and continuous use of drugs within the same chemical group (Waller, 1993; Torres-Acosta and Hoste, 2008). Taylor et. al. (2002) argued that failing to control internal parasites does not mean that AR has been developed. Using faulty drenching equipment and inaccurately estimating body weight can result in underdosing

and can contribute to failure to control GIN and development of AR. Underdosing promotes the accumulation of resistant alleles in a population by promoting survival of heterozygous resistant individuals (Jackson and Coop, 2000). Additionally, treating all animals at frequent and/or fixed intervals during stages of peak nematode transmission or treating the entire group when only a small percentage of animals exhibit clinical signs of parasitic infection are two management techniques that promote the development of AR (Kaplan et. al., 2004). The common management practices that promote AR are outlined in further detail in Table 2.3.

Waller (1993) stated that it is impossible for any anthelmintic to be 100% efficacious against 100% of parasite species 100% of the time. Therefore, when treating animals with anthelmintics, a small number of worms survive and become the most resistant of the population (Sangster, 1999). Surviving worms reproduce, contribute resistant genes to the next generation, contaminate pastures with resistant larvae, and gradually lead to the selection pressure of AR (Papadopoulos, 2008). The rate at which AR develops depends on those nematodes contributing to the next generation, which is measured as both the percentage of nematodes surviving treatment and the nematodes not exposed to the anthelmintics (Papadopoulos, 2008). Those nematodes not exposed to anthelmintics are described as being in refugia. Refugia include both nematode populations of untreated animals and the external phases of the nematode lifecycle including those present on pastures (Torres-Acosta and Hoste, 2008). By increasing the untreated population, resistance to anthelmintics can be slowed (Papadopoulos, 2008). Therefore, it is important that nematode control programs be designed to maintain the maximum amount of refugia while obtaining acceptable parasite control (Van Wyk, 2001).

Techniques available to detect AR on farms include a fecal egg count reduction test (FECRT), an egg hatch assay (EHA), and a larval development assay (LDA). The FECRT is the most popular technique (Torres-Acosta and Hoste, 2008). This *in vivo* test compares an animal's egg excretion before and after treatment with anthelmintics. Despite being a widely accepted method, this technique has its shortcomings. FECRT is labor intensive (Torres-Acosta and Hoste, 2008; Waller, 1997) and requires a second visit to the farm. Additionally, test results may not accurately estimate anthelmintic efficacy

Table 2.3. Promoting anthelmintic resistance through common management practices[1]

Management Practice	How Resistance is Promoted
Failure to quarantine new animals	New animals bring worms, and possibly AR nematodes, from previous location
Treatment of all animals in flock	Surviving worms infect pasture with resistant eggs and animals ingest resistant infective larvae
Underdosing	Use of benzimidazoles and levamisole especially increase AR
Extended use of the same family of anthelmintics	Repeated exposure of worms to same family of drugs results in a high selection pressure for resistant genes
Frequent treatment	Repeated exposure of worms to anthelmintics results in high selection pressure for resistant genes
Systematic treatment	Animals are treated at times irrelevant to the pattern of parasite infection. Instead they are treated to specific times for the animal (parturition, every 28 d, every 2 mo, etc.)
Inadvertent treatment	Animals are treated to control other parasites with a drug that also kills parasitic roundworms

[1]Adapted from Torres-Acosta and Hoste (2008).

because nematode egg output is not always correlated with the actual number of worms present. Instead, this test only measures the anthelmintic effect on egg production by mature worms (Taylor et. al., 2002). This test is also described as lacking sensitivity (Waller, 1997). Martin et. al. (1989) concluded that FECRT lacks the sensitivity to detect resistance levels below 25%.

Popular *in vitro* tests include the EHA and LDA. The EHA is especially accommodating in the detection of resistance to benzimidazole anthelmintics (Torres-Acosta and Hoste, 2008) as these anthelmintics prevent the embryonation and hatching of nematode eggs. Papadopoulos (2008) stressed the importance of using fresh feces (within 3 h of collection) since the sensitivity to thiabendazole will decrease with embryogenesis resulting in false negative results. Undeveloped eggs are incubated with serial concentrations of the anthelmintic and the percentage of eggs that hatch (or conversely die) at each concentration is determined. Similar to the FECRT, EHA lacks the sensitivity to detect resistance if the level is below 25% (Martin et. al., 1989).

The LDA method is described as being more laborious and time consuming than EHA (Papadopoulos, 2008). The advantage of this technique is that it measures AR to the major broad-spectrum anthelmintic groups, including macrocyclic lactones (Jabbar et. al., 2006). Macrocyclic lactones (avermectins and milbemycins) are products or chemical derivatives of soil microorganisms belonging to the genus *Streptomyces* (Vercruysse, 2005). Avermectins available for commercial use include ivermectin, abamectin, doramectin, eprinomectin and selamectin and commercially available milbemycins include milbemycin oxime and moxidectin. (Vercruysse, 2005). Additionally, this method requires only one farm visit to collect feces and if the producer is able to collect the fecal samples, this visit is not even required (Waller, 1997). A major advantage of this technique is that it allows the third stage larvae to be speciated, thereby, allowing nematode species to be identified in both the control and anthelmintic treatment (Papadopoulos, 2008). This particular test cultures first stage larvae into third stage larvae in the presence of a food source, heat treated lyophilized *Escherichia coli*, and the anthelmintic being tested (Coles et. al, 1988). Despite being labeled as laborious and time consuming (Jabbar et. al., 2006), the LDA test is believed to be more sensitive than

either the FECRT or EHA as it can detect AR when 10% of the worm population carries the resistant gene (Dobson et. al., 1996).

Certain management steps can be taken to prevent AR enhancement of nematodes. Previously, management required routine administration of anthelmintics every 3 to 4 weeks to control internal parasites (Malone, 1983). Researchers now realize this management practice has contributed to AR (Torres-Acost and Hoste, 2008). Today, reducing treatment frequency is considered the most important step to take to reduce the development of AR (Sangster, 1999; Jackson and Waller, 2008). Torres-Acosta and Hoste (2008) refer to past advice given to producers to use anthelmintics at the beginning of the wet season (to prevent a build-up of L3 larvae in fields), at the end of the wet season (to preventing the infestation from carrying over to the next wet season), and to treat animals with anthelmintics once in the dry season. Torres-Acosta and Hoste (2008) disagree with these previous recommendations because they directly compromise the refugia and select heavily for AR. Protecting the refugia is becoming a more important approach to preventing AR in order to maintain susceptibility within the suprapopulation (Pomroy, 2006), which refers to all of the parasite species in all stages of development within all hosts in an ecosystem. Reducing the drenching (deworming) frequency has the potential to reduce contamination with resistant phenotypes within the pasture. Additionally, extending the interval between treatments should allow susceptible phenotypes to become better established (Jackson and Waller, 2008).

Another management step to prevent AR, while maintaining refugia, includes selective treatment of animals within the flock because of disease susceptibility or their ability to promote infestation throughout the flock. This approach allows refugia to be maintained while administering treatment to only the animals requiring treatment. Use of the FAMACHA system can help to identify those animals requiring treatment. Leathwick et. al. (1999) supports this approach as they believe the role of an undrenched sheep on each farm is to provide a refuge for susceptible parasites, which can promote dilution of resistant parasites. Michel (1976) proposed the “dose and move” approach, which involved moving animals to a “clean” pasture (absence of L3 larva due to cultivation, reseeding, severe winter or dry season) immediately following administration of anthelmintics. Today, Torres-Acosta and Hoste (2008) conclude this approach both

selects heavily for AR and directly compromises refugia. They recommend moving animals to a clean pasture before administering treatment in order to protect the refugia.

Alternative Anthelmintic Treatments. Before commercial anthelmintics became available to producers, alternative products were available for use. The Department of Agriculture approved copper sulfate as an aid to sheep producers (Nighbert, 1932). Repeated testing over a 16-yr period showed, when properly administered, copper sulfate is both safe and 97% effective in controlling stomach worms (Nighbert, 1932). However, there is some disagreement among the literature concerning the recommended copper sulfate solution to use. A 1% solution is described as effective (Nighbert, 1932; Reed, 1930; Dimock, 1944) while Hardy and Schmidt (1932) described a 1.75 % solution against GIN. Kammlade and Kammlade (1955) recognized both solutions as controls of internal parasites.

Copper sulfate can be mixed alone or in combination with nicotine sulfate to form the cunic mixture. The cunic mixture offers additional parasite control against tapeworms (Dimock, 1944; Kammlade and Kammlade, 1955). However, Hardy and Schmidt (1932) described the cunic mixture as a "drastic treatment" that is not recommended for very weak animals. Kammlade and Kammlade (1955) agreed that the cunic mixture is a drastic treatment and cautioned that fatalities can occur if given in too large a dose. Instructions on preparation and dosage of both a 1% copper sulfate solution and the cunic mixture can be found in Kammlade and Kammlade (1955).

Present concern with using copper sulfate to control internal parasites is due to the sensitivity of sheep to high levels of copper. Death does occur if copper is consumed in excess (Torres-Acosta and Hoste, 2008). However, Hardy and Schmidt (1932) showed use of a 1.75 % solution of copper sulfate every 30 d for 4 yr did not cause any copper poisoning. This suggests the potential for copper sulfate to be used again to control intestinal parasites within flocks where worms exhibit AR.

Copper

Copper (Cu) is a trace mineral that is a dietary essential. It is distributed in muscle tissue, internal organs, blood, bones, and hair. In ruminant animals, over half of

the total Cu in the body is found in the liver (Davis and Mertz, 1987). Sheep have 10 times more liver Cu than pigs (Kline et. al., 1971; Davis and Mertz, 1987).

Functions. Copper is involved in necessary enzymatic activities leading to the formation of red blood cells and bone, myelination of the brain and spinal cord, and the production of hair and wool (Underwood and Suttle, 1999). Iron (Fe) and Cu apply essential catalytic function for post-translational biosynthesis and maturation of collagen and elastin, the major proteins of connective tissue (O'Dell, 1981). Lysyl oxidase is a Cu containing enzyme that initiates the covalent cross-linking of collagen and elastin in the extracellular space (Kagan and Li, 2003), providing structural rigidity and elasticity to the connective tissue and blood vessels (O'Dell, 1981). This occurs by hydroxylation of lysine residues in collagen and elastin (O'Dell, 1981). Cytochrome oxidase is the terminal component of the electron transport chain, which is present in all mammalian cells (Cromwell, 1997). This particular oxidase is essential to cellular respiration as it catalyzes the reduction of oxygen to water. Additionally, cytochrome oxidase is required for the normal myelination of both brain cells and the spinal cord, thereby, being necessary to maintain the integrity of the nervous system (Cromwell, 1997). Copper is believed to be a component of the polyphenyl oxidase (Pond et. al., 1995), which is required for normal hair and wool pigmentation. Polyphenyl oxidase catalyzes the conversion of tyrosine to melanin and incorporates disulfide groups into keratin in both wool and hair (Pond et. al., 1995). The disulfide groups provide cross-linkages of keratin resulting in normal keratinization of hair and wool (Cromwell, 1997). These disulfide linkages give curl to hair and crimp to wool.

Additional Cu containing enzymes include tyrosinase, superoxide dismutase, and ceruloplasmin. Tyrosinase is vital to the pigmentation process because it catalyzes the first two steps in the synthesis of melanin from tyrosine (Miller et. al., 1988). When this enzyme is deficient, the synthesis of melanin is reduced, resulting in a depigmentation of hair and skin (Cromwell, 1997). Copper is also involved in the disposal of likely damaging superoxide anions through the enzyme superoxide dismutase (Cromwell, 1997). This enzyme catalyzes the dismutation of monovalent superoxide anion radicals into hydrogen peroxide and oxygen, which is an important step in protecting cells from

the highly reactive free radicals that were generated by cellular metabolism (Miller et. al., 1988). An oxidase with an extensive specificity is ceruloplasmin. It is primarily involved in transporting Cu throughout the blood. Copper also acts as a free radical and superoxide ion scavenger by oxidizing Fe stored in the liver in the ferrous state (Fe^{2+}) to the ferric (Fe^{3+}) state. Oxidation is required for Cu to be bound and transported in the blood in order to regulate hepatic Fe mobilization (Frieden, 1980).

Copper Requirements: The Cu requirements of sheep are influenced by the levels of molybdenum (Mo) and sulfur (S) in the diet and the sheep's stage of production (lactation, gestation, breeding, growth). These minerals form insoluble complexes with Cu, thus reducing its absorption (SID, 2002). The daily allowance of Cu is dependent on the amount of Mo in the diet. The interaction between the daily allowance of Cu, Mo, and stage of production is given in Table 2.4.

Based on observations in the field, Dick and Bull (1945) concluded that Mo may act to limit the assimilation of Cu or decrease Cu storage in the liver of herbivorous animals. Therefore, they conducted an experiment using 30 two-toothed ewes (1 to 2 years of age) selected from a line of 40 crossbred ewes. Ewes were randomly subdivided into six groups of five ewes based on Cu concentration in the liver measured from previous samples taken by biopsy. Ewes consumed a diet of equal parts of oaten and lucerne chaff. Three groups had Cu added to the diet in the form of copper sulfate. Two of the three groups also received Mo at 10 mg or 100 mg/hd/d. Two other groups received Mo at the same levels without Cu and the final group acted as the experimental control receiving the basic diet of equal parts oaten and lucerne chaff. Groups receiving Cu were given 30 mg of Cu/ hd/d for the first 3 mo of the experiment. However, researchers acknowledged at some undetermined point in the second 3 mo the level of Cu was reduced to 3 mg/hd/d, not allowing them to determine the total amount of Cu added to the diet. At the end of the 6 mo trial, all ewes were slaughtered and the liver Cu concentration determined. These workers concluded that increasing Mo intake by sheep fed a normal diet by 10 or 100 mg/hd/d resulted in reduced ($P<0.10$) liver Cu concentration. Additionally, they concluded that an increase in the Mo concentration in pastures could explain the Cu deficiency in sheep grazing pastures, which show a forage

Table 2.4. Recommended daily copper allowance [a]

	Recommended Cu Allowance, ppm of diet DM		
Diet Mo, ppm	**Growth**	**Gestation**	**Lactation**
<1.0	8 – 10	9 – 11	7 – 8
>3.0	17 – 21	19 – 23	14 – 17

[a](SID, 2002).

Cu concentration within normal limits.

Based on results from research conducted during the latter part of the 1940's and early 1950's, Dick (1952) continued his research on the quantitative relationships between Cu and Mo. Thirty, two-toothed (1 to 2 years of age) crossbred ewes were subdivided into five groups based on liver Cu concentrations. Ewes were fed mixtures of chaffed oaten and lucerne hay. Copper sulfate and ammonium molybdate were added to the diet as sources of supplemental Cu and Mo. The daily intakes of Cu and Mo were 15 mg and 10.7 mg, respectively, across diets. At the end of the trial six months later, ewes were slaughtered and livers retrieved. Diets fed and liver Cu analyses at the beginning and end of the trial are given in Table 2.5. Although a slight elevation in liver Cu content was found in ewes consuming lucerne chaff (30 to 39 mg total liver Cu), there was an increase in Cu storage as the proportion of oaten chaff in the diet increased. Additionally, the levels of Mo in the blood are given in Table 2.6. At the conclusion of this experiment blood Mo levels were higher as the level of oaten chaff in the diet increased. Previously Dick and Bull (1945) demonstrated Mo either decreased or eliminated Cu storage in the liver when sheep consumed a diet of equal portions lucerne chaff and oaten hay. However, in this experiment Dick (1952) showed the nature of the diet plays an important role with regard to the amount of Cu storage in the liver.

In an effort to identify the "factor" in the previous experiments that was responsible for lowering the blood Mo level as the amount of oaten hay in the diet decreased, Dick (1953) focused on isolation of this substance and its interaction with inorganic sulfate. Through a series of experiments, Dick (1953) was able to determine the "factor" in lucerne chaff responsible for high Mo excretion was soluble in water, but insoluble in alcohol. After ashing 9 kg of lucerne chaff to further analyze the inorganic constituents, he showed solids of the watery ash extract were potassium carbonate, chloride and sulfate. To determine the effects of these salts on Mo excretion, the watery ash extract was put through an anion exchange resin column to remove the anions and stripped with potassium chloride to collect the sulfate in one fraction. The sulfate portion was the only one affecting Mo. Dick concluded when sulfate is low, Mo absorbed from the diet accumulates in the blood and tissues with little excreted in the urine. However, when sulfate intake is higher, more Mo is excreted in the urine, thus preventing

Table 2.5. Liver copper of sheep fed different ratios of chaffed oaten and lucerne hay[a]

	Liver Copper							
	Dry weight, ppm				Total liver copper, mg			
Experimental Diets	**Pre-experi-mental**	**After 6 months**	**Increase**	**S. E.**	**Pre- experi-mental [b]**	**After 6 months**	**Increase**	**S.E.**
Lucerne	212	299	87	± 38	30	39	9	± 5
Lucerne 3: Oaten 1	208	372	164	± 52	28	47	19	± 6
Lucerne 1: Oaten 1	213	497	284	± 37	27	62	35	± 4
Lucerne 1: Oaten 3	212	826	614	± 73	25	95	70	± 9
Oaten	208	1300	1092	± 139	26	139	113	± 18

[a]Adapted from Dick (1952).
[b]Calculated on estimated mean liver weight.

Table 2.6. Mean blood Molybdenum values for sheep fed various ratios of chaffed oaten and lucerne hay[a]

Experimental Diets	Blood molybdenum, ug/100 ml
Lucerne	48
Lucerne 3: Oaten 1	96
Lucerne 1: Oaten 1	180
Lucerne 1: Oaten 3	378
Oaten	447

[a]Adapted from Dick (1952).

accumulation in the blood. Based on the results of this experiment, it is now apparent that sulfate intake was responsible, in previous experiments (Dick, 1952), for increasing Mo levels in the blood when higher levels of oaten chaff was consumed. Although Dick could not determine if sulfate intake was responsible for Mo limiting Cu storage in ewes fed only lucerne, he did hypothesize the control of Cu storage by Mo is dependent on the supply of dietary sulfate.

Relationships among Cu, Mo, and sulfate have been demonstrated numerous times since Dick first reported the limiting effect of Mo on liver Cu storage. Copper absorption in lambs consuming a semi-purified diet was cut in half with the addition of as little as 2-4 mg Mo/kg DM (Suttle, 1983). Goodrich and Tillman (1966) studied the interrelationships of Cu, Mo, and S in a randomized block design with a 2 x 3 factorial arrangement of treatments. Levels of Cu, Mo and S were 10 and 40 ppm, 2 and 8 ppm, and .10 and .40%, respectively, of purified diet DM fed to 40 Rambouillet lambs. These researchers concluded that feeding .40% S as sulfate or 8 ppm Mo decreases the level of liver Cu. Additionally, feeding 40 ppm of Cu as coppper carbonate will increase liver Cu storage. Tables 2.7 and 2.8 illustrate the interaction among dietary Cu, S, and Mo and liver Cu storage in lambs of this study.

Sheep are responsive to the level of Cu in the diet. When levels become deficient, anemia, neonatal ataxia, loss of wool pigmentation in dark wool breeds, and loss of keratinization (crimp) of the wool are severe consequences (Underwood and Suttle, 1999). Anemia, due to Cu deficiency, is described as hypochromic. In this syndrome, a reduction in hemoglobin content causes red blood cells to appear pale and micocytic, causing in a reduction in the size of erythrocytes (Cromwell, 1997). Anemia occurs as the animal cannot incorporate iron into hemoglobin when Cu is deficient (Cromwell, 1997). Neonatal ataxia is a nervous condition in lambs more commonly referred to as swayback (Underwood and Suttle, 1999). This condition results in uncoordinated movements of the hind limbs and is caused by myelin aplasia (Barlow, 1963). Lambs born with this condition are unable to get up and nurse. Consequently, a high mortality rate is typical (Underwood and Suttle, 1999). All of these conditions are preventable with correct supplementation with Cu.

Table 2.7. Liver copper concentration, ppm DM[a]

	% Sulfur		
	.10	.40	
Cu level, ppm			
10	136.2	92.1	**
40	223.4	117.0	NS

[a]Adapted from Goodrich and Tillman (1966).
**P < 0.01.

Table 2.8. Liver copper concentration, ppm DM[a]

	Mo, ppm		
	2	8	
Cu level, ppm			
10	135.5	92.8	**
40	193.9	146.4	*

[a]Adapted from Goodrich and Tillman (1966).
*P < 0.05.
**P < 0.01.

Copper toxicity. Copper toxicity can occur in all animal species, although the most common form of Cu toxicity in farm animals is chronic Cu poisoning in sheep (Ross, 1966; Adamson et. al., 1969; Davis and Mertz, 1987). Todd (1969) described two types of Cu toxicity, acute and chronic. Acute Cu toxicity occurs when a single large dose acts as a corrosive poison, which causes enteritis, scouring, severe abdominal pain and possible death (Todd, 1969). Phase one of chronic Cu toxicity involves the accumulation of Cu in the liver over a period of weeks or months with no clinical symptoms present. The second phase is called "hemolytic crisis" and the most common characteristic involves the animal becoming dull, refusing to eat, and expressing excessive thirst. Mucous membranes reveal jaundice and hemoglobinuria becomes a major feature. Death, a common consequence of Cu toxicity can occur within a few hours, but generally will occur in 2 to 4 days (Todd, 1969).

The main organs targeted include the liver and kidney. Both the kidney and liver will darken in color when Cu toxicity occurs (Cromwell, 1997). Studies conducted by Ishamel et. al. (1971) used eight lambs given continuous doses of copper sulfate. Histological and histochemical changes of the liver were evaluated by needle biopsy. Details of the amount of Cu administered and amount of Cu found in the tissues *post mortem* can be found in Table 2.9. Table 2.10 describes the morphological changes observed from the liver biopsies. As Cu accumulates in the liver, both parenchymal cells and Kupffer cells swell and necrosis occurs resulting in focal necrosis of liver tissue (Ishmael et. al., 1971).

As Cu begins to accumulate in the body, serum enzyme levels can be used as indicators. During the first phase of chronic Cu toxicity, Cu starts accumulating in the tissues along with a significant rise in serum transaminase (Todd, 1969). This occurs several weeks before the development of hemolytic crisis. Serum glutamic oxaloacetic transaminase (SGOT), formerly referred to as aspartate aminotransferase (AST) (Cardinet III, 1989), has been proposed for early detection of Cu toxicity (Todd and Thompson, 1963; Ross, 1966; Davis and Mertz, 1987). According to Keen and Graham (1989), a rise in serum AST occurs during Phase 1 of Cu toxicosis. Alanine aminotransferase (ALT) is a liver specific indicator of damage that is well established and sensitive (Kramer, 1989). Additionally, a sudden sizeable rise in serum creatine phosphokinase

Table 2.9. Details of copper poisoned sheep[1]

	Sheep ID	Time of death from start of experiment, d	Total amount of $CuSO_4.5H_2O$ administered, g *	Calculated Cu Administered, g	Amount of Cu in tissues *post mortem*, ppm DM		
					Liver	Kidney	Spinal Cord
Copper poisoned	79	31	22	7.5	1730.3	288.7	5.5
	80	70	50	17.1	3864.7	384.2	8.8
	81	57	41	14.0	3194.1	343.8	7.2
	82	85	61	20.8	3486.5	534.4	8.5
	83	35	24	8.2	4064.3	303.6	4.0
	84	88	64	21.9	1640.8	304.0	4.8
	85	86	62	21.2	3168.5	290.0	8.5
	86	53	39	13.3	2653.4	435.3	4.2
Control	87	85	0	0	162.2	12.5	4.6
	88	86	0	0	189.9	7.4	2.4
	89	91	0	0	132.5	8.0	3.8
	90	91	0	0	217.9	10.5	3.4

[1]Ishmael et. al. (1971a).
*34.16% Cu.

Table 2.10. Morphological changes observed from liver biopsies taken from copper poisoned sheep before hemolytic crisis[1]

Biopsy	Vacuolation and fat droplets in parenchymal cells	Swelling of parenchymal cells	Necrosis of isolated parenchymal cells	Enlargement and vacuolation of parenchymal cell nucli	Swelling of isolated Kupffer cells	Clusters of swollen Kupffer cells.
Pre-dosing	7/7	0/7	0/7	0/7	0/7	0/7
3 wks Post-dosing	8/8	5/8	5/8	2/8	5/8	1/8
5 wks Post-dosing	6/6	5/6	5/6	5/6	5/6	3/6
7 wks Post-dosing	4/4	4/4	4/4	3/4	4/4	3/4
9 wks Post-dosing	1/1	1/1	1/1	1/1	1/1	1/1

[1]Ishmael et. al. (1971a).

levels, also known as creatine kinase (CK), suggests changes happening in the membranes of muscle cells as well as the kidney, liver, and brain. Normal AST, ALT, and CK values for sheep are 49 to123, 15 to 44, and 7.7 to 101 u/L, respectively (Boyd, 1984). Serum AST, ALT, and CK target either the liver or kidney. Therefore, monitoring the serum levels of these enzymes should identify animals developing Cu toxicosis.

Copper Metabolism. The sensitivity of sheep to Cu, both in deficit and excess, has been previously discussed. A knowledge of Cu metabolism is essential to appreciate this sensitivity in sheep. The regulation of Cu in the body and its delivery to cells must be carefully managed. Copper exists in two redox states. These states fluctuate by accepting or donating electrons (Hill and Link, 2009). As a result, Cu must be bound to a protein to prevent free radical production. Thus, being a required nutrient, Cu presents challenges to the body. An overview of Cu metabolism was provided by Cousins (1985). Copper is absorbed from the stomach and small intestine of the nonruminant (Cousins, 1985) and the abomasum of the ruminant (Turner et. al., 1987). Further, Cousins (1985) said Cu is transported in portal plasma bound mainly to albumin and possibly as amino acid complexes. In ruminant animals, Cu is absorbed from the abomasum, small intestine and colon (Turner et. al, 1987). Uptake by the liver occurs through a saturable transport process. Ceruloplasmin is responsible for the systemic transport of Cu from the liver and appears to donate Cu to tissues. The level of ceruloplasmin circulating in the blood can increase in response to “various stresses and disease related processes”.

According to Davis and Mertz (1987) Cu is absorbed by two mechanisms. When dietary Cu concentrations are low, Cu is transported mainly by the saturable or active pathways. In contrast, when dietary Cu concentrations are high, diffusion occurs. Turner et. al. (1987) used ligation of the rumen, reticulum, abomasum, small intestine at several points along the entire length, colon, and cecum in ewes in order to identify regions of the gastrointestinal tract where Cu absorption occurs. They detected Cu in venous blood within 5-min of injecting the Cu solutions in the abomasum, small intestine, and colon. No absorption was detected in the rumen or cecum after a 40-min period. Histological examination of the tissues indicated no damage to the mucosal linings with the

concentrations of Cu used. Therefore, they concluded, with the exception of the rumen and cecum, a wide area of the gastrointestinal tract is capable of absorbing Cu. Researchers determined Cu was absorbed by its presence in the bloodstream. With the absence of mucosal damage, they believed Cu was absorbed because it was not gaining entry via damaged tissues. Using everted sacs of hamster intestine, Campton et. al. (1965) determined the absorption of Cu was not the result of simple diffusion. Instead, they concluded a specific mechanism will transfer Cu from the cell to the bloodstream at specific sites of the cell surface or within the cell. Turner et. al. (1987) also evaluated the effect of including L-histidine, L-lysine, or L-glutamine to an *in vitro* incubation of a 6-m section of everted intestine divided into sacs between double ligatures placed at 15-cm intervals and found the absorption of Cu did not differ when histidine, lysine or glutamine were present. Instead, Cu absorption followed the same kinetic pattern as the absorption of ionic Cu. Based on results obtained, the absorption of Cu appears to be a two-stage process, Cu uptake from the lumen to a mucosal cell and, then, a transfer from the mucosal cell into the bloodstream (Turner, 1987).

In order to be transported across the brush border of the small intestine, Cu is bound to an absorbable ligand (Cousins, 1985). To facilitate its transfer into the portal circulation, Cousins (1985) suggested that Cu could be regulated by intracellular metallothionein (MT) concentrations. Metallothionein is a low molecular weight (6,500) metal binding protein present in most tissues and cell types (Bremner, 1987). The structure of metallothionein consists of 30% cysteine residues, which allow it to participate in the binding of copper. Cousins (1985) theorized the regulation of MT synthesis in mucosal cells could be influenced by intracellular Cu and/or Zn levels. If this were true, an excess of Cu in the mucosal cells would stimulate MT synthesis, MT would bind to Cu, and prevent it from being transported into the portal blood. Therefore, Cu bound to MT in the mucosal cells would be lost in normal cell turnover. Bremner (1987) disagreed with Cousin's theory for the regulation of Cu. He argued the mucosal cell synthesis of MT is not stimulated by "physiological" concentrations of Cu because the majority of the research on induction of MT synthesis by Cu has involved injection of pharmacological amounts of the metal (1 to 3 mg/kg BW). When given these amounts, Cu is as effective as other metals, including Zn, for increasing liver MT or MT mRNA

levels. However, at lower doses, Cu does not stimulate MT mRNA synthesis in mouse liver (Durnam and Palmiter, 1981).

After reviewing the MT potential to regulate Cu absorption, Cousins (1985) concluded that binding to MT in the gut mucosa was necessary to restrict the further translocation of Cu. Copper will bind to MT in the intestinal mucosa thereby preventing diffusion across the digestive tract layers into the portal blood (NRC, 2005). However, not all copper will bind to metallothionein at the intestinal mucosa, resulting in diffusion across the intestinal serosa and into the portal blood.

Cater and Mercer (2005) believe two proteins, which may be involved in the absorption of Cu, its transfer across the brush border into the enterocytes, and its uptake across the apical surface of the intestinal mucosa, are Crt1 and DMT1. Crt1 is a constantly encountered, high affinity transmembrane protein involved in Cu transport. DMT1, also referred to as nramp, is a divalent metal transporter protein located in the brush border (Cater and Mercer, 2005). DMT1 is known to be involved in the cellular uptake of Cu as well as Fe, Zn, and Mn. It is not known if DMT1 is involved in the regulation of Cu uptake when Cu is limited or when other trace elements are not present (Cater and Mercer, 2005). Crt1 is described as a protein having three transmembrane regions rich in histidine and methionine that allow Crt1 to bind with Cu (Hill and Link, 2009).

Once Cu has been absorbed, it can bind to albumin (Davis and Mertz, 1987; Keen and Graham, 1989; Linder and Hazegh-Azam, 1996; Underwood and Suttle, 1999), amino acids, or the transcuprein protein in order to be transported to the liver (Cromwell, 1997), the main storage organ of Cu. Copper bound to albumin has been reported as available to most tissues, although, the majority of Cu is taken up by the hepatocytes of the liver (Keen and Graham, 1989; Linder and Hazegh-Azam, 1996). The role of albumin in Cu transport is described as passive (Linder et. al., 1998). Albumin carries a large portion of exchangeable Cu in circulation before releasing it to other carriers for cell-specific uptake (Linder et. al., 1998). Transcuprein is theorized to be a possible carrier as it rapidly exchanges with Cu on albumin (Weiss and Linder, 1985).

After Cu reaches the liver, it can be incorporated into ceruloplasmin, bound to metallothionein (MT), or excreted via bile (Pond et. al., 1995). Binding with

ceruloplasmin allows Cu to be carried from the liver to the target organs (Davis and Mertz, 1987). As previously stated, translocation of Cu bound to MT is restricted and Cu cannot diffuse into the portal blood. Therefore, a significant amount of ingested Cu, bound to MT, is excreted in the feces during mucosal cell turnover (Davis and Mertz, 1987; NRC, 2005). The amount of Cu excreted in bile depends on the dose of Cu delivered. With larger doses, more Cu is excreted in the bile and vise versa (Linder et. al., 1998).

In sheep, slight modifications in the metabolism of Cu result in extreme sensitivity to the level of Cu in the diet. Corbett et. al. (1978) attempted to identify some of the differences in the intracellular distribution of Cu in the livers of normal, or representative sheep, and how the distribution of Cu is altered as a response to subacute dietary excess. This work compared sheep to rats due to the rat's insensitivity to Cu toxicity (Corbett et. al., 1978) and sheep being the most sensitive to Cu poisoning of all domestic animals (Saylor and Leach Jr., 1980; Underwood and Suttle, 1999). Milne and Weswig (1968) identified the insensitivity of rats to Cu as the ability to maintain constant concentrations of hepatic Cu when dietary levels of Cu are increased to 200 ppm. In this experiment, Corbett et. al. (1968) used eight male Long-Evans hooded rats fed a commercial diet containing 15.2 ppm of Cu to represent a source of normal rat liver, and eight crossbred yearling wethers randomly allotted to either the control (8.35 ppm Cu) or experimental (104.95 ppm Cu) diets. The researchers believed the experimental diet would elevate liver Cu without causing acute Cu toxicity: This was confirmed by a slight decrease in body weight. Animals consumed their diets for 23 d before being harvested and tissue samples collected. Results are given in Table 2.11. When the Cu status is normal, approximately half of the Cu in the cells of the rat liver is found in the cytosol fraction (Gregoriadis and Sourkes, 1967). Additionally, as the cellular Cu content increases, the percentage of Cu bound in the cytosol should decrease and the debris and large granule fractions of the liver should increase. However, the sheep liver presented a different pattern of intracellular Cu distribution. When comparing the cytosol fraction of the rats and control sheep, the sheep liver was lower ($P<0.01$) than normal rat. Also, the debris fraction of sheep contained a higher ($P<0.05$) percentage than the normal rat. The debris fraction represents the accumulation of Cu in the liver and the decrease in the

Table 2.11. Copper distribution among liver fractions of rats and sheep[a]

Species	Liver fractions, % total hepatic copper			
	Debris	Large granule	Microsome	Cytosol
Rat	12.8[b]	20.7[b]	12.7	53.9[b]
Control Sheep	37.0[c]	37.7[c]	9.9	15.4[c]
Copper-supplemented sheep	34.5[c]	24.9[b]	11.3	29.3[d]

[a] Adapted from Corbett et. al. (1978).
[b,c,d] Means within columns followed by different superscripts differ ($P<0.05$).

cytosol fraction, when comparing the rats and sheep, may suggest sheep lack the ability to synthesize cytoplasmic binding proteins resulting in copper accumulation in the liver.

Nevertheless, copper is necessary for numerous functions throughout the body. Therefore, great care should be given when providing copper to sheep.

Objectives

Due to the complexity of the gastrointestinal nematode *Haemonchus contortus* and the development of anthelmintic resistance to commercial dewormers, alternative methods for controlling this parasite are critical. The overall goal of this study was to accomplish the following:

1. Develop a Hampshire ewe flock that depend only on $CuSO_4$ to control *Haemonchus contortus* infestation.
2. Determine if $CuSO_4$ administration produces any toxicity effects evidenced by elevated levels of serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), and creatine kinase (CK) in Hampshire ewes.

CHAPTER III

Use of Copper Sulfate to Control *Haemonchus contortus* Infestation in Hampshire Ewes

Introduction

Normal lambing periods of Hampshire ewes in Kentucky occur in January and February. Lambs are typically weaned at an average age of 60 d. Typically, ewes are dewormed at weaning before they are sent out for maintenance on pasture. Signs of internal parasite infestation of ewes in confinement are uncommon, but if signs are present ewes are likely heavily infested. The objective of this study was to develop a Hampshire ewe flock that depended only on $CuSO_4$ to control *H. contortus* infestation.

Materials and Methods

Lambing and Pre-weaning: March, 2007. The timeline of Experiments 1 and 2 conducted over a 3-yr period can be found in Table 3.1. This chapter will focus primarily on Experiment 1. Hampshire ewes to lamb in January and February, 2007 were brought into the barn in December, 2006 where they remained until lambs were weaned. Ewes consumed 0.57 kg of a grain mix (Table 3.2) plus 2.27 kg of equal parts alfalfa haylage and corn silage until parturition. Ewes and newborn lambs were confined to lambing pens (jugs) for bonding for 2 to 5 d, after which they were assigned to mixing pens and provided a daily ration of 1.36 kg of a grain mix (Table 3.2) plus 4.08 kg of equal parts alfalfa haylage and corn silage per ewe until weaning.

Some Hampshire ewes exhibited edema (bottle jaw), a sign of internal parasite infestation, during the latter part of lactation. All ewes (59) were evaluated on March 27, for gastrointestinal parasite infestation based on FAMACHA scores, blood serum packed cell volumes (PCV), and fecal egg counts (FEC). The FAMACHA score is a method used to evaluate the level of anemia of each ewe (Figure 3.1). This technique compares the color of the mucous membranes of the lower eyelid against a card of predetermined colored scores ranging from 1 to 5. The more red in color, the lower FAMACHA score and, therefore, the less likely the animal is suffering from anemia (Van Wyk and Bath,

Table 3.1. Time table of experiments 1 and 2

Date	Experiment	Event	No. Ewes			
			FAMACHA	PCV	FEC	$CuSO_4$
03/30/07	1	Weaning	59	59	59	-
04/01/07	1	Drench	-	-	-	29
04/09/07	1		59	59	59	-
07/30/07	1	Begin Flushing Study	82	82	82	-
08/03/07	1		-	-	-	4
08/16/07	1	13 d post-drenching	82	82	82	-
08/17/07	1		-	-	-	12
11/02/07	1		71	-	-	24*
01/12/08-02/17/08	1	Lambing Period	-	-	71	71
02/06/08-03/03/08	1	12 d post-lambing	-	-	71	-
03/14/08	1	Weaning	14	14	14	3
03/27/08	1	Weaning	42	42	42	13
04/03/08	1	Weaning	13	13	13	6
03/26/08	1	12 d post-weaning	14	14	14	-
04/08/08	1	12 d post-weaning	42	42	42	5
04/15/08	1	12 d post-weaning	13	13	13	-
07/23/08	2	Begin Toxicity Trial	84	84	84	-
07/30/08	2	Drench	-	-	-	42
08/11/08	2	12 d post-drenching	84	84	84	-
08/13/08	2	Drench	-	-	-	13
08/25/08	2	12 d post-drenching	81	81	81	-
08/27/08	2	Drench				7
09/08/08	2	12 d post-drenching	7	7	7	-
11/11/08	1		71	71	71	4
11/18/08	1	Drench	-	-	-	8
11/24/08	1	13 d post-drenching (11/11/08)	4	4	4	-
12/01/08	1		71	12	12	-
01/05/09-02/12/09	1	Lambing Period	71	-	71	71
01/23/09-02/16/09	1	12 d post-lambing	71	-	71	-
03/13/09	1	Weaning	41	41	41	1
03/27/09	1	Weaning	27	27	27	-
03/25/09	1	12 d post-weaning	36	36	36	1
04/08/09	1	12 d post-weaning	27	27	27	-
05/01/09	1		76	-	-	4

*Ewes received Cydectin as a drench.

Table 3.2. Ingredient composition of grain mix fed in late gestation and lactation

Ingredient	Percent
Ground Shelled Corn	81.8
Soybean Meal (48% CP)	10.0
Distillers Dried Grains with Solubles	5.0
Southern States Sheep Mineral [a]	2.5
Ammonium Chloride	0.5
Vitamin E (IU/lb) [b]	0.125
Vitamin A, D, and E [c]	0.05

[a]SS Sheep Mineral contains 22.25% Ca; 6.00% P; 23.50% NaCl; 1.00% Mg; 1.00% S; 30 ppm I; 6 ppm Co; 32 ppm Se; 1,800 ppm Zn; 1,500 ppm Mn; 302,000 IU Vit. A/lb; 25,000 IU Vit. D/lb; and 200 IU Vit. E/lb.
[b]20,000 IU/lb.
[c]Vitamin A = 4,000,000 IU/lb; Vitamin D_3 = 800,000 IU/lb; Vitamin E = 500 IU/lb.

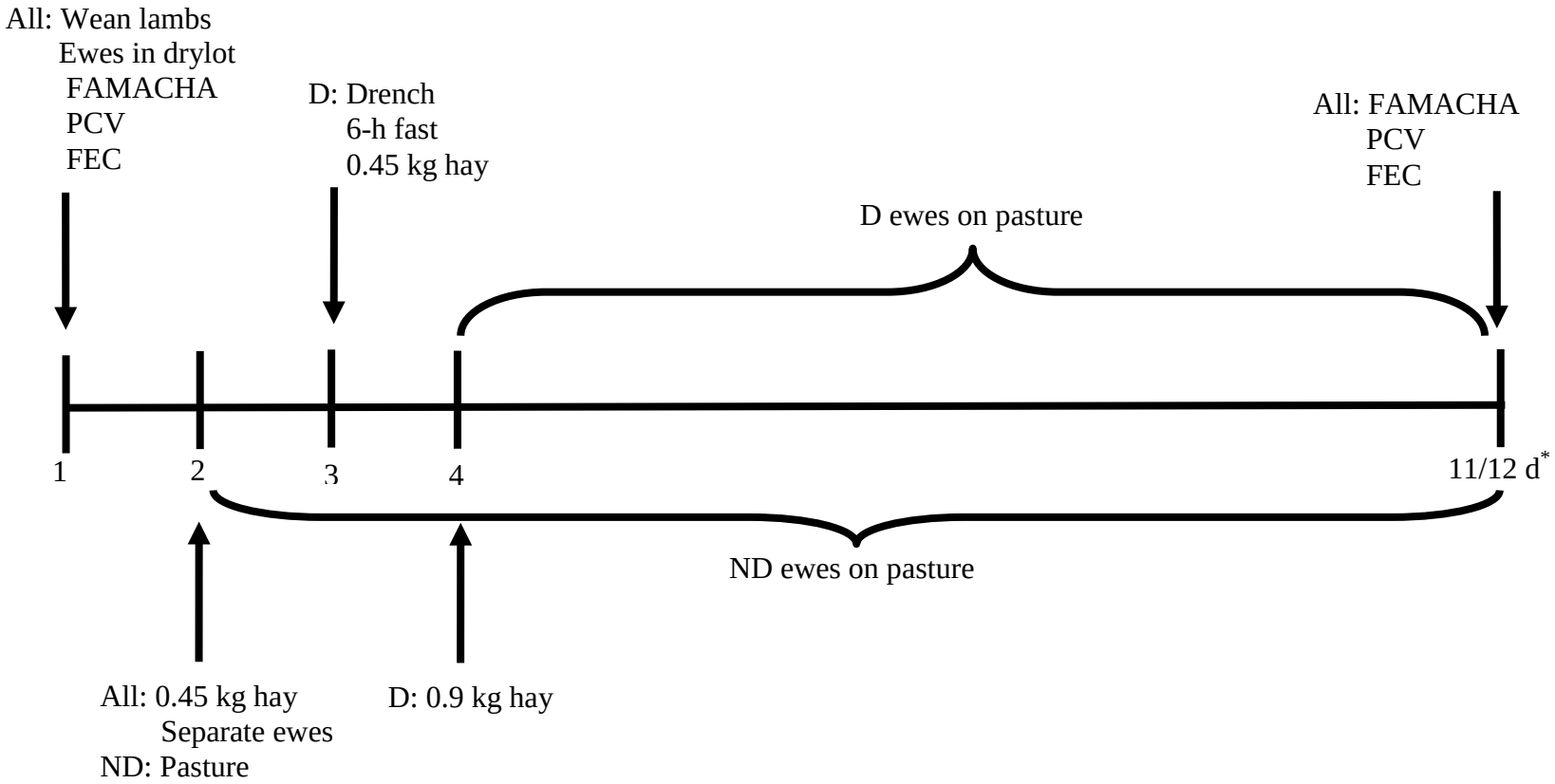
Figure 3.1. Assessing the FAMACHA score of Hampshire ewes.[1]

[1]Photo courtesy of Dr. Debra Aaron, University of Kentucky (2008).

2002). A blood sample was collected from each ewe by jugular venipuncture, centrifuged in a StatSpin multi-purpose centrifuge (StatSpin Technologies, Norwood, MA), and PCV was read from a Critspin digital microhematocrit reader (StatSpin Technologies, Norwood, MA). Rectal fecal samples were collected manually and analyzed for strongyle nematode egg concentration (eggs/g) by the modified McMaster technique (Whitlock, 1948). A subsample of 2.0 g of fresh feces was mixed with 28.0 g of a sugar solution made from equal parts sugar and water (v/v). The resulting slurry was strained through a coarse sieve (tea strainer). A 0.15-ml aliquot of filtrate was added to each grid of a McMaster counting slide (Paracount-EPG, Olympic Equine Products, Issaquah, USA) with each egg counted representing 50 eggs/g of feces. Each chamber of the McMaster counting slide holds 0.15 ml (total of 0.30 ml of fecal mixture). This is $1/100^{th}$ of the total volume of 30 ml (2.0 g feces + 28.0 g sugar solution). The number of eggs counted is multiplied by 100 and then divided by the 2.0 g feces to yield the total number of eggs/g feces. Fecal egg counts are not assumed to be normally distributed; thus, they were log transformed (FEC + 50) prior to analysis.

Weaning: March, 2007. On March 30 (d 1), lambs were weaned and ewes placed in a drylot without feed or water for 24 h following the timeline shown in Figure 3.2. On d 2 (March 31), all ewes were fed 0.45 kg/hd of grass hay at 0800 h before separating into drenched (D) and nondrenched (ND) groups. FAMACHA and PCV, along with an arbitrary FEC number, were used to distinguish ewes to be drenched versus those not drenched. This arbitrary number was 6,000 eggs/g feces and was developed from previous samplings which revealed as FEC exceeded 6,000 eggs/g feces, PCV decreased and FAMACHA scores increased. Twenty-nine ewes had FEC greater than 6,000 eggs/g (av. = 10,308) and remained in drylot without feed that evening. The remaining 30 ewes with FEC less than 6,000 eggs/g feces (ND) were turned to pasture (av. = 3,414). On d 3(April 1), the D ewes were drenched with a 1% copper sulfate/water solution ($CuSO_4$) at 0800 h, fasted for 6 h, and fed 0.45 kg/hd of grass hay at 1400 h. Research conducted by Ali and Hennessy (1996) has shown that feed restriction at or around the time of treatment with anthelmintics improves efficacy of dewormers. The 1% $CuSO_4$ solution

Figure 3.2. Experimental timeline: weaning 2007, 2008, and 2009.



*11 d in 2007; 12 d in 2008 and 2009.

was prepared by dissolving 28.4 g of $CuSO_4$ in 2,850 ml of water in a plastic container. Each ewe received 100 ml of the 1% solution. The D ewes were offered 0.9 kg/hd of grass hay at 0800 h on d 4 before joining the ND ewes on pasture. Both groups of ewes remained on pasture until d 11 (April 9) when they were re-evaluated for FAMACHA, PCV and FEC.

Post-weaning: 2007. On July 30, ewes were FAMACHA scored and blood and feces collected for PCV and FEC analyses, respectively, prior to a 2-wk nutritional flushing period. Four ewes were drenched with $CuSO_4$ on August 3 (av. FEC = 5,013). Ewes were re-evaluated for FAMACHA, PCV, and FEC on August 16. Twelve ewes received the 1% solution of $CuSO_4$ on August 17, based on FEC greater than 6,000 eggs/g (av. = 7,363).

Rams were removed on September 28 and ewes were sheared on October 12. FAMACHA scores were determined on November 2. Seventy-one of the 82 ewes that were nutritionally flushed were brought into the lambing barn on December 21 and vaccinated for enterotoxemia and tetanus (with CDT antitoxin). Ewes remained in the barn and were offered a late gestation diet (Table 3.1) before parturition.

Lambing: 2008. Lambing began on January 12, 2008, and continued until February 17. Seventy-one ewes were fecal sampled and drenched with a 1% solution of $CuSO_4$, on an average date of January 30, 2008, as they left the lambing pen (2 to 5 d post partum). All 71 ewes were fecal sampled again between February 6 and March 3 (av. = February 16, 2008).

Weaning: March, 2008. One hundred sixteen Hampshire lambs were weaned at an average of 59 d on either March 14, March 27, or April 3, 2008. At weaning, 69 ewes were sampled and grouped as described for weaning in March, 2007 and illustrated in Figure 3.2. Fourteen ewes were sampled on March 14, 42 ewes on March 27, and 13 on April 3. Twenty-two ewes (av. FEC = 9,477) were drenched with $CuSO_4$ after they were first sampled at weaning (three on March 14, thirteen on March 27, and six on April 3). The 14 ewes initially sampled on March 14 were re-sampled on March 26 (12 d post-

weaning). All 14 ewes had FEC < 6,000 eggs/g feces therefore, no ewes were drenched with $CuSO_4$. The 42 ewes sampled at weaning on March 27 were re-sampled on April 8 (12 d post-weaning). Thirty seven of the 42 ewes had FEC < 6,000 eggs/g and did not receive $CuSO_4$. The other five ewes were drenched the same day (April 8). Three of the five had received $CuSO_4$ after sampling at weaning (av. FEC d 0 = 13,167 eggs/g; av. FEC d 12 = 9,767). On April 3, 13 ewes were sampled at weaning and then re-sampled 12 d later (April 15). All 13 ewes sampled had FEC < 6,000 eggs/g. Therefore, none of these ewes were drenched with $CuSO_4$.

Toxicity Study (Summer, 2008). Eighty-four Hampshire ewes were involved in Experiment 2, beginning July 23. Ewes were FAMACHA scored and blood serum PCV and FEC determinations were made. Ewes were randomly allotted, based on age and July 23 FEC, into D ($CuSO_4$ drenched) or ND (not drenched). Jugular blood samples were collected at pre-determined intervals post-drenching in vacutainer tubes. Following centrifugation at 1000 RPM for 10 min, serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), and creatine kinase (CK) were analyzed as indicators of Cu toxicity. This study is described in Chapter IV.

Summer, 2008: Immediately following the toxicity trial, seven ewes with FAMACHA scores ≥ 4 or FEC exceeding 6,000 eggs/g feces in the ND treatment were drenched on August 13 with $CuSO_4$ (av. FAMACHA = 4; av. FEC = 4,129). Six ewes in the D group (av. FAMACHA = 4; av. FEC = 1,108) were drenched at the same time. Twelve days after drenching (August 25), 81 ewes were re-sampled for FAMACHA, PCV, and FEC. Two days later, four ewes from the ND group (av. FAMACHA = 4; av. FEC = 4,538) and three ewes from the D group (av. FAMACHA = 4; av. FEC = 14,783) were drenched with $CuSO_4$. These ewes were evaluated for FAMACHA, PCV, and FEC again 12 d later (September 8). The average FAMACHA score of both treatment groups was 3. The FEC for ND and D ewes were 2,375 and 633 eggs/g feces, respectively.

Pre-lambing: 2008. Seventy-one ewes were evaluated for FAMACHA and samples were collected for PCV and FEC analyses on November 11, 2008. Four ewes were drenched

on the same day because their FAMACHA scores were 4. Eight ewes were drenched 7 d later (November 18) based on November 11 FEC exceeding 6,000 eggs/g feces (av. = 8,519). The four ewes drenched on November 11 were re-evaluated for FAMACHA, PCV and FEC 13 d later (November 24). Seventy-one ewes were FAMACHA scored on December 1. These included the 12 ewes previously drenched in November (four on November 11 and eight on November 18). Only the 12 ewes previously drenched on November 11 and 18 were evaluated for PCV and FEC. The average PCV of these 12 ewes was 30.0. Average FEC was 4,489. None of the 71 ewes received any drench until post-lambing.

Lambing: 2009. Seventy-one Hampshire ewes lambed during the period from January 5 to February 12. Each ewe received $CuSO_4$ as they exited the lambing pen (2 to 5 d post-partum). A FAMACHA score was recorded and a fecal sample obtained for FEC analysis at the same time. Ewes were re-evaluated for FAMACHA and re-sampled for FEC analysis an average of 12 d after leaving the lambing pens.

Weaning: 2009. One hundred thirteen Hampshire lambs were weaned at an average age of 60 d on March 13 or March 27 from 68 ewes. At weaning, ewes were sampled and grouped as described for March, 2007, and 2008, and as illustrated in Figure 3.2. Forty-one ewes were sampled at weaning on March 13. Based on FEC, one ewe was drenched (FEC = 9,000 eggs/g). Thirty-six ewes from the group weaned on March 13 were re-sampled on March 25 (12 d post-weaning). During this time period five ewes were culled. The same ewe drenched on March 13 was re-drenched following sampling on March 25. On March 27, 27 ewes were sampled at weaning for FAMACHA, PCV and FEC. No ewes were drenched. Twenty-seven ewes were re-checked for internal parasite infestation 12 d later on April 8. No ewes required $CuSO_4$.

Post-weaning: 2009. Seventy-six Hampshire ewes were evaluated for FAMACHA score before exposure to rams in May, 2009. Any ewe with a FAMACHA score of greater than 3 was drenched with $CuSO_4$ (av. FAMACHA = 3). Four ewes received $CuSO_4$.

Statistical Analysis. FAMACHA, PCV, FEC and log-transformed FEC data collected from all ewes (D and ND) at weaning on days 0 and 11 (2007) or 0 and 12 (2008) as well as the respective changes from Day 0 to Day 11 (or 12) were analyzed using PROC GLM of SAS (Windows version 5.1/2600, SAS Inst., Inc., Cary, NC). The statistical model included effects of treatment (D and ND) and ewe age (2, 3, 4, 5, 6, and 7). Analyses were conducted by year. The effect of ewe age was not significant in any year; therefore, those means are not presented. Also, the effect of treatment on actual and log-transformed FEC data was similar so actual FEC means are presented. Differences between FAMACHA scores (2009) and FEC (2008 and 2009) collected from drenched ewes as they left the lambing jugs were analyzed using paired t-tests. Statistical significance was indicated at the $P < 0.10$, 0.05, and 0.01 levels.

Results and Discussion

Pre-weaning and Weaning: March 2007. Fifty-nine Hampshire ewes were evaluated for FAMACHA score, and sampled for PCV and FEC analysis on March 30 based on an observed external sign of internal parasite infestation, i.e., bottlejaw. The presence of bottle jaw was surprising because sheep maintained in confinement typically do not have significant levels of the stomach worm *H. contortus* because pasture forage is necessary for this parasite to complete its lifecycle (Hempworth et. al., 2006). The University of Georgia College of Veterinary Medicine, Department of Infectious Diseases, had diagnosed *H. contortus* as the predominant worm species in the University of Kentucky sheep flock in 2006 (R. M. Kaplan, University of Georgia, Athens, GA, personal communication). Tied with this finding were assays for the level of resistance of the stomach worms to common commercial anthelmintics. Results showed a high resistance to Benzimidazoles (Panacure, Safeguard, Valbazen) and Levamisole (Tramisole and Levasole), resistance to Ivermectin (Ivomec, Eprinex, and Dectomax), and a low resistance to Moxidectin (Cydectin) (R. M. Kaplan, University of Georgia, Athens, GA, personal communication). The efficacy of Moxidectin was estimated at 80 to 95%, compared with 0% and 52% for Benzimidazoles and Levamisole, respectively (R. M. Kaplan, University of Georgia, Athens, GA, personal communication). The efficacy of

Ivermectin was not available. The fear of encountering a resistance to the overuse of Cydectin, began a search for alternative methods of controlling stomach worms in the University Hampshire flock.

A search through the literature found several control methods that have been tried and/or used successfully and/or unsuccessfully during the period from the 1930's until the 1970's, when commercially manufactured dewormers came on the market (Williams, 1997). Old-time remedies for controlling infestation include garlic (Waller, 1999), lead arsenate (Hebden, 1962), tetrachlorethylene (Kammlade and Kammlade, 1955), phenothiazine (Thorp et. al., 1945; Kammlade and Kammlade, 1955), and a cunic mixture ($CuSO_4$ and nicotine sulfate). Nigtbert (1932) tested the use of $CuSO_4$ over a 16-yr period and found its proper use was both safe and 97% effective in controlling stomach worms. When nicotine sulfate is combined with $CuSO_4$ (cunic) both stomach and tapeworms have been controlled (Kammlade and Kammlade, 1955). The DrenchRite test, by the University of Georgia, indicated the University of Kentucky's predominant worm species was the *H. contortus* 96% of the time (R. M. Kaplan, University of Georgia, Athens, GA, personal communication). This indicated the use of nicotine sulfate was unnecessary in the University of Kentucky flock. Therefore, the recommendation of Kammlade and Kammlade (1955) for the preparation and administration of a 1% $CuSO_4$ solution was adopted.

Twenty-nine ewes were drenched with a 1% solution of $CuSO_4$ on April 1, 2007, based on their FEC exceeding 6,000 eggs/g feces. The average FAMACHA, PCV, and FEC values for d 0 and d 11 are given in Tables 3.3, 3.4, and 3.5 respectively. The FAMACHA scores for D were higher than ND ewes on Day 0 ($P < 0.01$) and Day 11 ($P < 0.10$). Although FAMACHA scores of both groups decreased numerically from d 0 to d 11 (-0.4 and -0.1 for D and ND, respectively), the magnitude of this change was not large enough to be statistically significant. The PCV of D ewes were lower than ND both pre- and post-drenching (Table 3.4). The PCV of D ewes increased 4.7 units (18%) as the ND ewes increased 2.8 units (9%) from d 0 to d 11. These increases were different ($P < 0.05$). The FEC of the D group was higher ($P < 0.01$) on d 0 than ND. However, $CuSO_4$ administration decreased the FEC of D ewes to the level of ND ewes so differences on d 11 were nonsignificant (2,041 vs. 2,209). The decrease in FEC of D

Table 3.3. FAMACHA scores of $CuSO_4$ drenched and nondrenched ewes at weaning and 11 days later (2007)[a]

	Treatment[b]		
Item	D	ND	P-value
Number ewes	29	30	
Day 0	3.6	2.9	0.01
Day 11	3.2	2.8	0.10
Change	- 0.4 (-11)[c]	- 0.1 (-3)[c]	NS

[a]1 = bright red; 5 = white.
[b]D = drenched; ND = no drench.
[c]Percent change from day 0 to 11.

Table 3.4. PCV percentages of blood serum collected from $CuSO_4$ drenched and nondrenched ewes at weaning (2007)

	Treatment [a]		
Item	D	ND	P-value
Number ewes	29	30	
Day 0	26.3	30.2	0.01
Day 11	31.0	33.0	0.10
Change	+ 4.7 (+18)[b]	+ 2.8 (+9)[b]	0.05

[a]D = drenched; ND = no drench.
[b]Percent change from day 0 to 11.

Table 3.5. FEC of $CuSO_4$ drenched and nondrenched ewes at weaning and 11 days later (2007)[a]

	Treatment[b]		
Item	D	ND	P-value
Number ewes	29	30	
Day 0	10,308	3,414	0.01
Day 11	2,041	2,209	NS
Change	-8,267 (-80)[c]	-1,205 (-35)[c]	0.01

[a]Eggs/g feces.
[b]D = drenched; ND = no drench.
[c]Percent change from day 0 to 11.

ewes from d 0 to d 11 (-8,267 eggs/g; -80%) was greater ($P < 0.01$) than ND ewes (-1,205 eggs/g; -35%). Multiple experiments conducted by Rietz (1935) between the years of 1930 to 1933 involved drenching multiple groups of sheep with various levels (3 or 4.5 oz.) of either a 1% or 1.5% $CuSO_4$ solution in 21 d intervals. Fecal samples were collected before treatment and 2 wk following drenching with $CuSO_4$ for FEC. Results were similar to the findings of the current study, in all instances; $CuSO_4$ reduced nematode ova of the treated animals.

Research conducted by Vanimisetti and coworkers (2004) measured bodyweight, FEC and PCV of ewes ranging in age from one to ten years of age at weaning. Ewes were de-wormed with levamisole before lambing and once post-weaning. One week after de-worming all ewes were dosed with 10,000 L3 larvae of *H. contortus*. Body weight, FEC and PCV were measured at dosing, and 3, 5, 7, 9, and 11 wk post infection. Ewes were maintained on pasture and could have been continuously exposed to GIN. Overall, ewes did not lose weight. Ewes lambing in the spring showed an increase in logFEC gradually until 9 wk and then a dramatic decrease. PCV decreased through wk 9. This does not agree with the data collected in this study. Both ewes in the D and ND groups showed a decrease in FEC and an increase in PCV. Differences could be due to the effectiveness of levamisole as a drench when compared to $CuSO_4$ and the load of internal parasites ewes were carrying between the two studies.

Post-weaning: 2007. FAMACHA scores of the four ewes identified to drench (D) with $CuSO_4$ on July 30 was initially 2.75. The four ewes received $CuSO_4$ on August 3. Thirteen days post-drenching, scores of these ewes were 2.0. The lower the FAMACHA score, the lower the level of internal parasites of the animal as indicated by a brighter red mucous membrane of the eye. When comparing the FAMACHA scores of the four D ewes to the 78 that were not drenched (ND), these scores averaged 2.75 compared with 2.94 for the 78 ewes. In this case, the decision to drench four of 82 ewes was not based entirely on FAMACHA scores. Instead, FAMACHA scores were combined with PCV's and FEC's to arrive at an arbitrary decision. The four ewes drenched on August 3 had higher FEC on average when compared to the remaining 78 ewes (avg FEC = 5,013).

The FAMACHA scores of ND ewes decreased to 2.60 as the score for the four D ewes decreased to 2.0. The PCV of the four D ewes was lower initially than ND (D = 21.8 vs. ND = 30.3). However, the PCV determined 13 d later (August 16), was 27.4% (+5.6 units) for D ewes and 29.8% (-0.5 units) for ND ewes. Again, an increase in PCV value suggests less blood removal and therefore, a decrease in the number of GIN. Drenching with $CuSO_4$ may have reduced the GIN level and allowed ewes to begin to recover from their day 0 anemic condition. The FAMACHA change of the four ewes heavily infected with GIN is substantiated by the increase in serum PCV concentration. The average initial FEC of D ewes were 5,013 whereas the FEC of ND ewes was only 871 eggs/g feces. The average FEC of D ewes decreased 2,000 eggs/g feces as the ND ewes increased 2,617 eggs/g feces during the 13 d from August 3 to August 16. The numerical decrease (less anemia) in FAMACHA score, increase in PCV, and decrease in FEC in the D ewes indicate that $CuSO_4$ administration did have an effect on the GIN infestation of these four ewes.

The effectiveness of both $CuSO_4$ as a drench and FAMACHA scores, used in conjunction with PCV and FEC, is illustrated by how few ewes required drenching post weaning 2007. Malan et. al. (2001) credit the FAMACHA system with reducing the number of ewes drenched. By using the FAMACHA system drenching was reduced by 90% (Malan et. al., 2001).

Lambing: 2008. All 71 ewes that lambed were drenched with $CuSO_4$ (1% solution; 100 ml) and fecal sampled as they left the lambing jugs to enter the mixing pen of ewes and newborn lambs. Results are shown in Table 3.6 The average FEC was 2,189 eggs/g feces. All ewes were re-sampled at an average of 12 d post-drenching. The average FEC had significantly decreased ($P < 0.001$) to 1,170 eggs/g feces. Previous research has determined fecal egg output to increase dramatically 2 weeks before and up to 8 weeks post-lambing (Gibbs, 1984; Courtney et. al., 1984). This increase in FEC of lactating ewes was determined the “periparturient” rise by Crofton (1958). Ewes in the current study were drenched at lambing in order to allow for the periparturient rise in fecal egg counts in lactating ewes.

Table 3.6. FEC of ewes drenched with 1% $CuSO_4$ solution as they left the lambing jug (D 0) and 12 d later (2008)[a]

	FEC[b]
Day 0	2,189
Day 12	1,170

[a]n = 71.
[b]Day 0 vs. 12 (P < 0.001).

Table 3.7. Changes in FAMACHA, PCV, and FEC of $CuSO_4$ drenched and nondrenched ewes at weaning (2008)

	Treatment[a]			
Item	D		ND	
Number ewes	22		47	
FAMACHA				
Day 0	2.73		1.85	
Day 12	2.86		2.64	
Change	+0.13	(+5)[c]	+0.79[b]	(+43)[c]
PCV				
Day 0	25.7		29.8	
Day 12	26.0		30.2	
Change	+0.3	(+1)[c]	+0.4	(+1)[c]
FEC, eggs/g feces				
Day 0	9,477		2,720	
Day 12	2,414		1,834	
Change	-7,063[b]	(-75) [c]	-886	(-33)[c]

[a]D = drenched; ND = drenched.
[b]Day 0 vs. 12 (P<0.001).
[c]Values in parentheses = percent change from Day 0 to 12.

Weaning: March, 2008. Sixty-nine of the 71 ewes that left the lambing jugs weaned lambs from March 14 to April 3, 2008. Lambs averaged 59 days of age at weaning. Changes in FAMACHA, PCV and FEC of 69 of these ewes are shown in Table 3.7. Lambs of two of the original ewes that left the lambing jugs died prior to weaning. Consequently, these ewes were removed from the flock during lactation and were not sampled at weaning. Drenched (D= > 6,000 eggs/g feces) and ND (< 6,000 eggs/g feces) groups were established from initial FEC taken at weaning. Initial FAMACHA scores of the 22 D ewes (2.73) were higher initially than the 47 ND ewes (1.85). The FAMACHA of D ewes increased only 0.13 units from Initial (D 0) to Final (D 12) whereas ND ewes increased 0.79 units. PCV's of D ewes were lower initially when compared to ND ewes (25.7 vs. 29.8). Only a minute, non-significant, change was found from the Initial to the Final sampling. The FEC of D ewes was higher initially than ND ewes (D = 9,477 vs. ND = 2,720). While FEC of both groups decreased post-drenching, the decrease was significantly greater ($P < 0.001$) in the D ewes (-7,063 eggs/g; -75%) than ND ewes (-886 eggs/g; -33%). Drenching with $CuSO_4$ decreased the FEC in D ewes to a level that was similar to that of ND ewes within 12 days afterwards (D = 2,414 vs. ND = 1,834 eggs/g feces). Woolaston (1992) observed increased FEC in lactating ewes and theorized the increase to be due to the stress of lactation. Reduction in FEC 12 d post-weaning of ewes in this study agree with research conducted by McAnulty and coworkers (2001). Removal of lambs from lactating ewes resulted in decrease in egg counts.

Summer: 2008. Eighty-four Hampshire ewes participated in a $CuSO_4$ drench toxicity trial prior to breeding. This experiment is discussed in Chapter IV. Fifty-two of the 84 ewes weaned lambs previously and 32 yearlings were added.

Pre-lambing: 2009. Twelve ewes of 71 were drenched with $CuSO_4$ after assessment of FAMACHA score, PCV, and FEC on November 11, 2008 as all ewes prepared to enter the last 4 to 6 weeks of gestation. The 12 ewes deemed needing drenching had initial FAMACHA scores, PCV values and FEC of 2.7, 28.0% and 9,079 eggs/g feces, respectively. Concurrent initial data for 59 ND ewes were 1.6, 31.5%, and 2,074 eggs/g feces. When ewes were re-sampled 13 d later on November 24, 2008, the FAMACHA

scores of the D and ND groups were the same as previously (D = 2.7 vs. ND = 1.6). Only the 12 ewes previously drenched were re-sampled for PCV and FEC on November 24. The PCV of these 12 ewes increased to 30.1 and FEC decreased to 4,489 eggs/g feces.

Lambing: 2009. All ewes received $CuSO_4$ and were evaluated for FAMACHA score and a fecal sample taken for FEC as they exited the lambing jugs 2 to 5 d post-lambing. Changes in FAMACHA score and FEC are shown in Table 3.8. The average FAMACHA score and FEC was 1.8 and 1,537 eggs/g feces, respectively. No ewes had FAMACHA scores greater than 3 (av. = 1.6) or FEC exceeding 6,000 eggs/g feces (av. = 521) when sampled 12 d later. Therefore, no ewes received $CuSO_4$ after lambing in 2009.

Weaning: 2009. Sixty-eight of 71 ewes that lambed were evaluated at weaning for FAMACHA score and sampled for PCV and FEC analysis. One ewe of 41 sampled on March 13, was drenched with $CuSO_4$. Although her FAMACHA score was 2.0, her PCV was 25.5, and FEC was 9,000 eggs/g feces. The averages for the 67 ND ewes were 1.7, 29.7, and 856 eggs/g feces. None of these 67 had FEC's above 6,000. The only ewe to have more than 6,000 eggs/g feces was the ewe that had been previously drenched. Apparently, the $CuSO_4$ drench was ineffective in decreasing this isolated case of heavy stomach worm infestation. This ewe was drenched again on March 25 but was not re-sampled. Instead, this ewe was culled from the flock. Sheep flocks managed within the

FAMACHA system routinely cull ewes that have continually high FEC. Such ewes carry 70 to 80% of the eggs within a flock (Kaplan et. al., 2004), and therefore, are contaminators of ewes with lower levels of stomach worm infestations (Barger and Dash, 1987).

Table 3.8. Change in FAMACHA and FEC of ewes drenched with 1% $CuSO_4$ solution as they left the lambing jug (D 0) and 12 d later (2009)[a]

	FAMACHA	FEC[b]
Day 0	1.8	1537
Day 12	1.6[c]	521[c]

[a]n = 71.
[b]Eggs/g feces.
[c]Change ($P < 0.001$) between Day 0 and 12.

Summary

Identification of ewes that required drenching with a 1% $CuSO_4$ solution, throughout this 2-yr study, was based primarily on FEC's (> 6,000 eggs/g feces). Secondarily, FAMACHA scores and serum PCV's were evaluated. Continual analysis of these three variables revealed the number of ewes that required drenching at weaning decreased from 29 in 2007, 22 in 2008, and one in 2009. Of the 59 original ewes that began the study in 2007, 13 were in the flock at weaning in 2009. Ewes were culled for typical production reasons, as well as continually heavy stomach worm infestations. Simultaneously, yearling ewes were incorporated into the flock to replace the culls. The existing flock in 2009 appeared to be more resistant to stomach worms than in 2007. Reasons for this include culling ewes with chronic high levels of stomach worms and the use of $CuSO_4$ drench to keep infestations at manageable levels as measured from FAMACHA scores, PCV's, and FEC's.

CHAPTER IV

The Potential of Copper Sulfate to Cause Toxicity When Used as an Anthelmintic for Hampshire Ewes.

Introduction

The use of $CuSO_4$ as a drench for sheep was a popular recommendation to producers by extension agents in the 1950's. As more commercial dewormers became available in the 1960's and 1970's, its use was phased out. Now, as internal parasites have become more resistant to commercial drenches through mismanagement such as overuse of anthelmintics, underdosing, using faulty equipment, continuous use of one family of drugs, and drenching the entire flock when only a few animals require treatment, producers are beginning to search for alternative methods. One method could be drenching with $CuSO_4$. However, some producers are hesitant to use $CuSO_4$ due to the sheep's sensitivity to Cu. The objective of this study was to determine if $CuSO_4$ administration produced any toxicity effects evidenced by elevated levels of serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), and creatine kinase (CK) in Hampshire ewes.

Materials and Methods

Summer: 2008. Research protocols were approved by the University of Kentucky Institutional Animal Care and Use Committee. An overview of this trial can be found in Figure 4.1. To begin, 84 Hampshire ewes were sampled before breeding on July 23, 2008 for FAMACHA, PCV, and FEC as described in Chapter III. Of these 84 ewes, 20 had been in the flock since initial de-worming with $CuSO_4$ in March 2007. Ewes were blocked by age and balanced for FEC collected on July 23. Forty-two were assigned to a non-drenched treatment (ND) as the other 42 were designated to receive a $CuSO_4$ drench (D). The 42 allotted to the D treatment were drenched with 100 ml of a 1% solution of $CuSO_4$ before the initial blood (0 h) was collected at 0800 h on July 30. Jugular blood was collected (Figure 4.2) from all ewes at 2 (1000 h), 4 (1200 h), 8 (1600 h), and 12 h (2000 h) on the first day. After the 12-h collection, all ewes were fed 0.91 kg low-quality

Figure 4.1. Timeline of $CuSO_4$ toxicity trial.

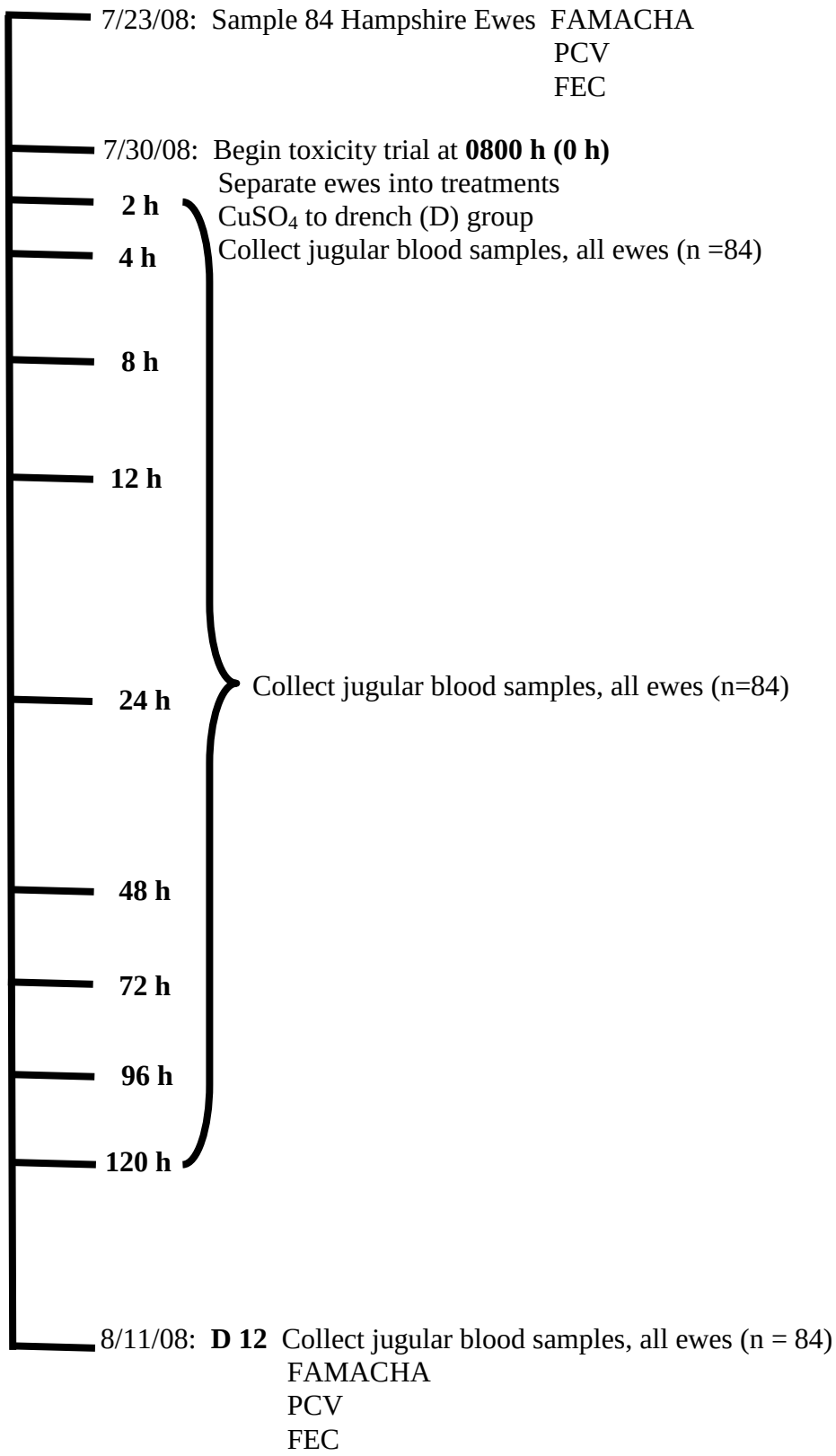


Figure 4.2. Collecting jugular blood samples from Hampshire ewes.[1]

[1]Photo courtesy of Dr. Debra Aaron, University of Kentucky (2008).

grass hay per head and remained in confinement overnight. Other blood samples were taken at 24, 48, 72, 96, and 120 h, as well as 12 d after the initial blood collection. Ewes returned to pasture immediately after blood was collected each time after the 2000 h collection (24, 48, 72, 96, and 120 h and 12 d post-drenching). While on pasture, ewes consumed 0.45 kg/hd of a grain mix (Table 3.2) at 0730 h daily. All ewes were re-evaluated for FAMACHA, PCV, and FEC on d 12 post drenching. Following blood collections on d 12, one fertile ram was placed with each group of 42 ewes confined in an open-sided barn.

All blood samples were collected into plastic 10-ml serum vacutainer tubes, immediately placed on ice and transported to an onsite laboratory. Fresh blood was centrifuged at 1000 RPM for 10 min at 22°C. Approximately 5 ml of serum was pipetted into 7-ml plastic scintillation vials and frozen at -80°C until analyzed.

Laboratory Analyses. Frozen serum samples were acclimated to room temperature before analyzed for serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), and creatine kinase (CK) using the ACE *Alera* clinical chemistry system (Alfa Wassermann Diagnostic Technologies, LLC, West Caldwell, NJ) at the University of Kentucky Veterinary Diagnostic Laboratory .

Upon analyzing serum samples at 0, 2, 4, 8, 12, 24 h serum AST, ALT, and CK fell within normal range (Boyd, 1984). For analysis efficiency, samples were analyzed at the halfway point (120 h) and 12 d later (288 h) in order to correspond to FAMACHA, PCV, and FEC also collected on d 12.

Experimental Design and Statistical Analysis. This experiment was conducted as a generalized randomized complete block design with a one-way treatment structure (D and ND). Ewes were blocked according to parity [0 (17 to 18 mo having never lambed), 1 (30 to 31 mo having lambed once), and 2 (42 mo or older having lambed 2 or more times)] and balanced for FEC collected on July 23. FAMACHA, PCV and log-transformed FEC data collected on July 23 (Initial) and 19 d later (Final) as well as the respective changes (Initial to Final) were analyzed using PROC GLM of SAS (Windows version 5.1/2600, SAS Inst., Inc., Cary, NC). FEC were log transformed prior to analysis

to induce normality. The statistical model included fixed effects of parity, treatment, and the parity x treatment interaction. Parity differences were determined using all possible t-tests (PDIFF option). Blood data were analyzed by collection time post-drench (2, 4, 8, 12, 24, 120, and 288 h) using the same model. In addition, initial AST, ALT, and CK values were used as covariates for the respective models. Statistical significance was indicated at the $P<0.10$, 0.05, 0.01 levels.

Results and Discussion

FAMACHA scores are shown in Table 4.1. No differences were found between D and ND groups on July 23 (Initial) or 19 d later on August 11 (Final). Although scores increased numerically in both treatments from the Initial to Final scoring, these increases were not statistically significant. Similarly, PCV values determined initially and 19 d later were not different (Table 4.2). The Final PCV values were numerically lower than initially, but the effect of drenching vs. non-drenching was not significant. Table 4.3 shows Initial logFEC was similar for D and ND treatments. However, the final logFEC of D ewes was lower ($P=0.013$) than ND. As a result, the logFEC increase was 0.32 for D whereas it was 0.69 for ND ($P<0.001$). The statistically low initial FAMACHA scores (2.26 to 2.35), high PCV values (33.62 to 34.09) and low logFEC (2.15 to 2.21) indicate neither group of ewes was suffering from a heavy load of stomach worms. These results could infer that drenching with $CuSO_4$ may be a sound management practice even if ewes are carrying a relatively small load of stomach worms.

Blood serum values for aspartate aminotransferase (AST) are presented in Table 4.4. Values in both treatments fell within the normal range (49 to 123 u/L) reported by Boyd (1984). There were no statistical differences between treatments from 2 to 288 h (12 d) post-drenching. The serum values for alanine aminotransferase (ALT) at different times after drenching are depicted in Table 4.5. Boyd (1984) reported a range of 15 to 44 u/L for serum ALT values in sheep. The values shown in Table 4.5 fall within this range and show no differences between D and ND ewes at any post-drenching time. Similar results were found for serum creatine kinase (CK) (Table 4.6). These values were more variable

Table 4.1. Least squares FAMACHA means of drenched (D) and nondrenched (ND) Hampshire ewes[a]

	Treatment			
Item	D	ND	SEM[b]	P-value
Initial (7/23)	2.26	2.35	0.101	0.547
Final (8/11)	2.55	2.73	0.123	0.305
Change	+0.29	+0.38	0.118	0.574

[a]n = 42 ewes per treatment.
[b]Standard error of mean.

Table 4.2. Least squares PCV means (%) of drenched (D) and nondrenched (ND) Hampshire ewes[a]

	Treatment			
Item	D	ND	SEM[b]	P-value
Initial (7/23)	33.62	34.09	0.564	0.563
Final (8/11)	31.48	31.24	0.558	0.754
Change	-2.14	-2.85	0.468	0.285

[a]n = 42 ewes per treatment.
[b]Standard error of mean.

Table 4.3. Least squares logFEC means of drenched (D) and nondrenched (ND) Hampshire ewes[a]

	Treatment			
Item	D	ND	SEM[b]	P-value
Initial (7/23)	2.21	2.15	0.083	0.594
Final (8/11)	2.53	2.84	0.088	0.013
Change	+0.32	+0.69	0.076	0.001

[a]n = 42 ewes per treatment.
[b]Standard error of mean.

Table 4.4 Least squares means of serum aspartate aminotransferase (u/L) in ewes at different time intervals after drenching with $CuSO_4$[a]

	Treatment[b]			
Item	D	ND	SEM[d]	P-value
Post-drench, h[c]				
2	90.2	89.4	1.05	0.574
4	88.0	88.2	0.80	0.860
8	86.9	85.9	0.80	0.363
12	86.3	85.9	0.92	0.764
24	87.8	85.6	1.80	0.386
120	78.4	77.9	1.35	0.783
288 (12 d)	86.7	88.5	1.92	0.516

[a]n = 42 ewes per treatment.
[b]D = drenched; ND = no drench.
[c]Average AST at 0 h = 88.3.
[d]Standard error of mean.

Table 4.5. Least squares means of serum alanine aminotransferase (u/L) in ewes from 2 to 288 hours post-drenching with $CuSO_4$[a]

	Treatment[b]			
Item	D	ND	SEM[d]	P-value
Post-drench, h[c]				
2	20.9	20.6	0.40	0.614
4	20.2	20.2	0.36	0.993
8	19.7	19.9	0.41	0.687
12	19.9	20.1	.0.32	0.657
24	19.9	19.5	0.47	0.549
120	18.7	19.3	0.28	0.159
288 (12 d)	19.6	20.2	0.41	0.334

[a]n = 42 ewes per treatment.
[b]D = drenched; ND = no drench.
[c]Average ALT at 0 h = 20.1.
[d]Standard error of mean.

Table 4.6. Least squares means of serum creatine kinase (u/L) in ewes from 2 to 288 hours after drenching with $CuSO_4$[a]

	Treatment[b]			
Item	D	ND	SEM[d]	P-value
Post-drench, h[c]				
2	176.1	193.5	34.1	0.719
4	147.7	126.1	13.4	0.256
8	131.0	112.0	8.02	0.098
12	139.4	124.7	9.93	0.300
24	124.9	121.8	9.25	0.816
120	107.7	121.0	17.5	0.593
288 (12 d)	110.7	124.9	8.61	0.248

[a]n = 42 ewes per treatment.
[b]D = drenched; ND = no drench.
[c]Average CK at 0 h = 143.3.
[d]Standard error of mean.

than AST and ALT values and some fell outside the range reported by Boyd (1984), but the only statistical difference (P = 0.098) between D and ND was found at 8 h post-drench. Todd (1969) described the first phase of Cu toxicity as an accumulation of Cu in the tissues that resulted in a significant rise in serum AST. Additionally, Todd and Thompson (1963), Ross (1966), and Davis and Mertz (1987) have proposed that increased levels of AST in serum is an early indicator of Cu toxicity. Furthermore, a large amount of CK is contained in both heart and skeletal muscle (Smith, 1972). Therefore, increases in serum CK activity indicates disease or damage to cardiac or skeletal muscle (Szasz et. al., 1976). Since no significant spike or any consistent difference between treatments was found for these enzyme levels after drenching, it is concluded that drenching Hampshire ewes with 100 ml of a 1% $CuSO_4$ solution (410 mg actual Cu) did not result in Cu toxicity.

All ewes were maintained in confinement overnight on July 30 and consumed an average of 0.9 kg low quality orchardgrass hay from an 2000 h feeding. Based on NRC (1984) values, ewes consumed an average of 18 mg from hay (20 ppm Cu) overnight. Ewes in the D group had received 410 mg earlier (0800 h) when they were drenched with 100 ml of 1% $CuSO_4$ solution, for a total Cu consumption of 428 mg. Once ewes (D and ND) returned to pasture, all consumed an estimated 10.2 mg Cu/d from orchardgrass forage (7 ppm). By the end of the toxicity trial on August 11, ND ewes had consumed a total of 140.4 mg Cu (10.2 mg/d x 12 d + 18 mg from hay). D ewes had consumed 550.4 mg Cu (140.4 mg via hay and pasture + 410 mg in 1% $CuSO_4$ drench). In earlier research by Ishmael et. al. (1971a), Cu poisoned sheep consumed 7,500 mg Cu over a 31-d period (242 mg/d) in order to cause death. In comparison, estimates of total Cu consumption for ND and D ewes of the present 12-d study were 140.4 and 550.4 mg, respectively (11.7 and 45.8 mg/hd/d). Combined with no significant differences between D and ND ewes for serum AST, ALT, and CK the total consumption of 550.4 mg Cu by D ewes over the 12-d period of this study is below a calculated 2,904 mg/wether lamb (242 mg x 12 d) used in the Ishmael et. al. (1971a) study.

Ewes in this study varied in age from 17 mo to 7 yr. Consequently, they varied in parity (number of previous lambings). But, they were blocked to treatment by age and balanced for July 23 FEC which allowed parity to be equally represented in both

treatments. Thirty-two ewes were 17 to 18 mo old and were assigned a parity of 0 when this toxicity study began. These ewes were subsequently bred and lambed first at 23 to 24 mo of age. Twenty-one ewes (30 to 31 mo) were assigned a parity of 1 and lambed for their second time at 35 to 36 mo. The remaining 31 ewes (42 to 43 mo and older) were assigned a parity of 2 and lambed approximately 5 mo later.

The effect of parity on FAMACHA scores, PCV values, and logFEC is illustrated in Table 4.7. Initial FAMACHA scores were lower and PCV values higher for ewes with a parity of 0 than for older ewes (parity 1 and 2). Differences between 1 and 2 parities for FAMACHA and PCV were nonsignificant. No parity differences were found for initial logFEC. Similar effects were found on August 11 (Final) with older ewes having higher FAMACHA scores and lower PCV values than younger ewes. Differences for logFEC were again nonsignificant. FAMACHA scores and PCV values of ewes in the 0 parity group changed the least from July 30 to August 11 (Initial to Final) whereas parity 2 ewes changed the most. No differences were found for logFEC.

These data differ from research by Hoste et. al. (2006), who conducted a study within flocks of dairy ewes to determine if either ewe age or level of milk production affected ewe response to gastrointestinal nematodes (GIN). Their study was conducted on three farms with each farm using three groups of 20 ewes. Twenty ewes were in their first lactation, while the two other groups used multiparous adult ewes which were either 3, 4 or 5 yr of age. The 40 multiparous ewes were subdivided into two groups of 20 each, based on level of milk production (high vs. low). Ewe response to GIN was based on fecal and blood samples taken four times per year (February, March, May, and September). Fecal samples were used to determine FEC and blood samples were used to determine the concentration of pepsinogen and inorganic phosphate in the serum, which reflect mucosal damage that can be an indicator of the size of the worm population of the abomasum (via pepsinogen) and small intestine (via inorganic phosphate). At the conclusion of the experiment, researchers found a higher excretion of eggs in the feces of the ewes in their first lactation when compared to the multiparous ewes. This was confirmed by statistical differences ($P<0.01$) of fecal egg excretion between farms 2 and 3. No statistical difference was found for GIN infestation between the low and high producing ewes on any farm.

Table 4.7. Effect of parity on least squares FAMACHA, PCV, and logFEC means

	Parity[a]			
	0	1	≥ 2	SEM[b]
FAMACHA				
Initial (7/23)	1.97[c]	2.39[d,e]	2.50[e]	0.124
Final (8/11)	2.09[c]	2.73[d,e]	3.10[e]	0.150
Change	+ 0.12[c]	+ 0.34[c,d]	+ 0.55[d]	0.143
PCV, %				
Initial (7/23)	35.20[c]	33.00[d,e]	33.35[e]	0.687
Final (8/11)	33.67[c]	30.65[d,e]	29.70[e]	0.680
Change	- 1.53[c]	-2.35[c,d]	-3.59[d]	0.570
logFEC				
Initial (7/23)	2.06	2.26	2.21	0.101
Final (8/11)	2.60	2.77	2.70	0.107
Change	+ 0.54	+ 0.51	+ 0.49	0.076

[a] 0 = 17-18 mo, 0 times lambing; 1 = 30-31 mo, 1 lambing; 2 = 42-43 mo or older, 2 or more lambings.

[b] Standard error of mean.

[c,d,e] Means within the same row with different superscripts differ (P<0.05).

A possible explanation for the differences between the Hampshire ewe data collected in this study and Hoste et. al. (2006) could be due to the difference in parity groups. Hoste et. al. (2006) did not have any ewes sampled before they lambed (parity 0). Ewes in the parity 0 of the current study were yearlings that had not lambed. Therefore, their body had not been stressed as older ewes in parities 1 and 2, so they should theoretically be more able to withstand a parasitic infestation. Moreover, the Hampshire ewes in parity 2 were composed of older ewes ranging in age from 3 to 7 years. Over the years, older Hampshire ewes who were less tolerant of parasitic infestation had been culled from the flock. Therefore, remaining ewes should be more tolerant to parasitic infestation (Fleming et. al., 2006). The data collected in the current study does correspond to data reported by Aaron et. al. (2009) who found an increase in parasitic infestation with increasing parity as indicated by FAMACHA score, PCV value, and logFEC in Polypay and White Dorper ewes.

Summary

Eighty-four Hampshire ewes used in a toxicity study were randomly allotted to treatments based on age and FEC from samples collected before breeding (July 23). The two treatments used were $CuSO_4$ drench (D) and no drench (ND). On July 30 at 0800 h (0 h), ewes were separated into treatment groups. Those in the D treatment were drenched with 100 ml of 1% $CuSO_4$ and a jugular blood sample was collected immediately afterwards. A jugular blood sample was also collected from ND ewes. Subsequently, blood serum was collected from each ewe at 2, 4, 8, 12, 24, 48, 72, 96, and 120 h and 12 d post-drenching (August 11). All blood samples collected at 0, 2, 4, 8, 12, 24, 120, and 288 h were analyzed for AST, ALT, and CK. Additionally, on d 12, ewes were FAMACHA scored and blood and feces collected for PCV and FEC analyses.

Results of this study showed no differences initially or 12 d post-drenching between D and ND groups for FAMACHA and PCV. Initial logFEC scores were similar between treatments. Final logFEC were lower for D ewes than ND ($P = 0.013$), despite an increase in logFEC in both groups. Blood serum AST and ALT concentrations did not differ between treatments at any collection time between 2 to 288 h (12 d) post-

drenching. Likewise, CK did not differ between treatments at any collection time, except 8 h post-drenching.

Conclusion

Phase one of chronic Cu toxicity involves Cu accumulation in the liver over a period of weeks or months without clinical symptoms being present. Indicators of Cu accumulation in the body include increased levels of serum AST, ALT and CK. Based on the evidence provided from this study, administration of 100 ml of a 1% $CuSO_4$ solution (410 mg actual Cu) can be used as a de-wormer to treat stomach worm infestations in sheep without creating any symptoms of Cu toxicity.

CHAPTER V

Summary

In the first experiment, $CuSO_4$ was used to control *Haemonchus contortus* infestation in Hampshire ewes at weaning over a 3 year lambing period from March 2007 until March 2009. At weaning 2007, ewes were FAMACHA scored and blood and feces collected for PCV analysis and FEC, respectively. Ewes were placed in drylot and fasted for 24 h. Those with FEC exceeding 6,000 eggs/g feces remained in confinement and were drenched with a 1% $CuSO_4$ solution. Ewes with FEC less than 6,000 eggs/g feces were turned out to pasture. Ewes were re-evaluated for FAMACHA score, PCV and FEC 12 days later. Overall, the FAMACHA scores and FEC of ewes drenched with $CuSO_4$ decreased, while PCV increased. These data indicate $CuSO_4$ is an effective dewormer.

In the second experiment, the potential of $CuSO_4$ to cause Cu toxicity was evaluated. Eighty-four Hampshire ewes were blocked to two treatments by age and balanced for July 23 FEC: ewes receiving $CuSO_4$ (D) and no $CuSO_4$ (ND). Jugular blood samples were collected from all ewes immediately after D ewes received $CuSO_4$ and at 2, 4, 8, 12, 24, 48, 72, 96, 120, and 288 h post-drenching. FAMACHA scores, PCV, and FEC were also evaluated at 288 h post-drenching. Jugular blood samples were evaluated for serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), and creatine kinase (CK). Above normal levels of these enzymes indicate liver degeneration, a common side effect of Cu toxicosis. After AST, ALT, and CK analyses, no elevated levels were detected and a reduction in PCV was found. Results of these data suggest the efficacy of $CuSO_4$ as a dewormer.

The two experiments reported in this thesis indicate $CuSO_4$ can be used to control *Haemonchus contortus* infestation in ewes. Additionally, the second experiment indicates $CuSO_4$ is safe and can be used without fear of causing Cu toxicosis in sheep when used as a de-wormer in a FAMACHA system.

CHAPTER VI

Implications

Due to the impact of *Haemonchus contortus* on the sheep industry and the widespread development of anthelmintic resistance, alternative methods of control must be found. The research conducted for this thesis has demonstrated the ability of $CuSO_4$ to be used in conjunction with the FAMACHA system, PCV, and FEC in order to control stomach worms in Hampshire ewes. Furthermore, $CuSO_4$ can be used as a dewormer without fear of causing Cu toxicosis in sheep even though they are sensitive to high levels of Cu.

LITERATURE CITED

Aaron, D. K., D. G. Ely, E. Fink, B. T. Burden, and M. M. Simpson. 2009. Factors affecting indicators of *Haemonchus contortus* infection in Polypay and percentage White Dorper ewes. J. Anim. Sci. 87 (E Suppl. 3):108. (Abstr.)

Adamson, A. H., D. A. Valks, M. A. Appleton, and W. B. Shaw. 1969. Copper toxicity in housed lambs. Vet. Rec. 85:368-369.

Aerts, R. J., T. N. Barry, and W. C. McNabb. 1999. Polyphenols and agriculture: beneficial effects of proanthocyanidins in forages. Agric. Ecosyst. Environ. 75: 1-12.

Ali, D. N. and D. R. Hennessy. 1996. The effect of level of feed intake on the pharmacokinetic disposition and efficacy of ivermectin in sheep. J. Vet. Pharmacol. Ther. 19:89-94.

Barger, I. A. 1997. Control by management. Vet. Parasitol. 72:493-506.

Barger, I.A. 1999. The role of epidemiological knowledge and grazing management for helminth control in small ruminants. Int. J. Parasitol. 29:41-47.

Barger, I. A. and K. M. Dash. 1987. Repeatability of ovine faecal egg counts and blood packed cell volumes in Haemonchus contortus infections. Int. J. Parasitol. 17:977-980.

Barlow, R. M. 1963. Further observations on swayback. I. Transitional pathology. J. Comp. Pathol. Therap. 73:51-60.

Barry, T. N., D. M. McNeill, and W. C. McNabb. 2001. Plant secondary compounds; their impact on nutritive value and upon animal production. Pages 445-452 in Proc. XIX Int. Grass. Conf., Sao Paulo, Brazil.

Bath, G. F. and J. A. Van Wyk. 2001. Using the FAMACHA system on commercial sheep farms in South Africa. Proceedings of the 5th International Sheep Veterinary Congress, 22-25 January 2001, Cape Town, South Africa.

Bown M.D., D. P. Poppi, and A. R. Sykes. 1986. The effect of post-ruminal infusion of protein or energy on the pathology of *Trichostrongylus colubriformis* infection and the body composition in lambs. Pages 27 in 46th Proc. of the New Zea. Soc. Of Anim. Prod., Dunedin, New Zealand.

Bown, M. D., D. P. Poppi, and A. R. Sykes. 1991. Nitrogen transactions along the digestive tract of lambs concurrently infected with *Trichostrongylus colubriformis* and *Ostertagia circumcincta.* Brit. J. Nutr. 66:237-249.

Boyd, J. W. 1984. The interpretation of serum biochemistry test results in domestic animals. Vet. Clin. Pathol. 13:7-14.

Bremner, I. 1987. Involvement of metallothionein in the hepatic metabolism of copper. J. Nutr. 117:19-29.

Cardinet III, G. H. 1989. Skeletal muscle function. Page 484 in Clinical Biochemistry of Domestic Animals. 4th ed. J. J. Kaneko ed. Academic Press, San Diego, CA.

Cater, M. A. and J. F. B. Mercer. 2005. Copper in mammals: Mechanisms of homeostasis and pathophysiology. Pages 101-129 in Molecular Biology of Metal Homeostasis and Detoxification. Topics in Current Genetics. No. 14. M. J. Tamás and E. Martinoia, ed. Springer Verlag, Berlin, Germany.

Coles, G. C., J. P. Tritschler II, N. J. Laste, and A. L. Schmidt. 1988. Larval development test for detection of anthelmintic resistant nematodes. Res. Vet. Sci. 45:50-53.

Corbett, W. S., W. W. Saylor, T. A. Long, and R. M. Leach, Jr. 1978. Intracellular distribution of hepatic copper in normal and copper-loaded sheep. J. Anim. Sci. 47: 1174-1179.

Courtney, C. H., C. F. Parker, K. E. McClure, and R. P. Herd. 1984. A comparison of the periparturient rise in fecal egg counts of exotic and domestic ewes. Int. J. Parasitol. 14:377-381.

Cousins, R. J. 1985. Absorption, transport and hepatic metabolism of copper and zinc: special reference to metallothionein and ceruloplasmin. Physiol. Rev. 65:238-309.

Crampton, R. F., D. M. Matthews, and R. Poisner. 1965. Observations on the mechanism of absorption of copper by the small intestine. J. Physiol. 178:111-126.

Crofton, H. D. 1958. Nematode parasite populations in sheep on lowland farms. V. Further observations on the post-parturient rise and a discussion of its significance. Parasitol. 48:251-260.

Cromwell, G. L. 1997. Copper as a nutrient for animals. Page 177-186 in Handbook of Copper Compounds and Applications. H. Wayne Richardson, ed. Marcel Dekker Inc., New York.

Davis, G. K. and W. Mertz. 1987. Trace Elements in Human and Animal Nutrition. Pages 301-350. 5th ed., Acad. Press, New York.

Dick, A. T. 1952. The effect of diet and of molybdenum on copper metabolism in sheep. Aust. Vet. J. 28:30-33.

Dick, A. T. 1953. The effect of inorganic sulphate on the excretion of molybdenum in the sheep. Aust. Vet. J. 29:18-26.

Dick, A. T. and L. B. Bull. 1945. Some preliminary observation on the effect of molybdenum on copper metabolism in herbivorous animals. Aust. Vet. J. 21:70-72.

Dimock, W. W. 1944. Some parasitic diseases of sheep: prevention and control. Circular 55. Kentucky Agric. Exp. Sta. Univ. of Kentucky, Lexington.

Dinaburg, A. G. 1944. Development and survival under outdoor conditions of eggs and larvae of the common ruminant stomach worm, *Haemonchus contortus.* J. Agric. Res. 69:421-433.

Dobson, R. J., L. Lejambre, and J. H. Gill. 1996. Management of anthelmintic resistance: inheritance of resistance and selection with persistent drugs. Int. J. Parasitol. 26:993-1000.

Durnam, D. M. and R. D. Palmiter. 1981. Transcription regulation of the mouse metallothionein-I gene by heavy metals. J. Biol. Chem. 256:5712-5716.

Ellenby, C. 1968. Desiccation survival of the infective larva of *Haemonchus contortus*. J. Exp. Biol. 49:469-475.

Fleming, S. A., T. Craig, R. M. Kaplan, J. E. Miller, C. Navarre, and M. Rings. 2006. Anthelmintic resistance of gastrointestinal parasites in small ruminants. J. Vet. Intern. Med. 20:435-444.

Frieden, E. 1980. Caeruloplasmin: a multi-functional metalloprotein of vertebrate plasma. Page 93-124 in Biological Roles of Copper. New York: Elsevier/North-Holland.

Gibbs, H. C. 1982. Mechanisms of survival of nematode parasites with emphasis on hypobiosis. Vet. Parasitol. 11:25-48.

Goodrich, R. D. and A. D. Tillman. 1966. Copper, sulfate and molybdenum interrelationships in sheep. J. Nutr. 90:76-80.

Gregoriadis, G. and T. L. Sourkes. 1967. Intracellular distribution of copper in the rat. Can. J. Biochem. 45:1841-1851.

Hardy, W. T. and H. Schmidt. 1932. Stomach worms in sheep and goats. Bulletin No. 448. Texas Agric. Exp. Sta., College Station.

Hebden, S. P. 1962. Recent advances in anthelmintics for use in sheep. Aust. Vet. J. 38:164-169.

Hempworth, K., M. Neary, and T. Hutchens. 2006. Managing internal parasitism in sheep and goats. Ext. Pub. No. AS-573-W. Purdue Univ., West Lafayette.

Hill, G. M., and J. E. Link. 2009. Transporters in the absorption of utilization of zinc and copper. J. Anim. Sci. 87(E. Suppl.): E85-E89.

Hoste, H., A. C. Rulie, F. Prevot, J. P. Bergeaud, C. Grisez, F. De La Farge, P. Jacquiet, and P. Dorchies. 2006. Differences in receptivity to gastrointestinal infections with nematodes in dairy ewes: Influences of age and of the level of milk production. Small Rumin. Res. 63:150-155.

Ishmael, J., C. Gopinath, and J McC. Howell. 1971. Experimental chronic copper toxicity in sheep: Histological and histochemical changes during the development of the lesions in the liver. Res. Vet. Sci. 12:358-366.

Jabbar, A., Z. Iqbal, D. Kerboeuf, G. Muhammad, M. N. Khan, and M. Afaq. 2006. Anthelmintic resistance: the state of play revisited. Life Sci. 79:2413-2431.

Jackson, F. and R. L. Coop. 2000. The development of anthelmintic resistance n sheep nematodes. Parasitol. 120:S95-S107.

Jackson, F., and P. Waller. 2008. Managing refugia. Tropical Biomedicine. 25:34-40.

Jasmer, D. P., R. B. Wescott, and J. W. Crane. 1986. Influence of cold temperatures upon development and survival of eggs of Washington isolates of *Haemonchus contortus* and *Ostertagia circumcincta*. Proc. Helminthol. Soc. Washington 53: 244-247.

Kagan, H. M. and W. Li. 2003. Lysyl oxidase: properties, specificity and biological roles inside and outside of the cell. J. Cell. Biochem. 88:660-672.

Kammlade, Sr., W. G. and W. G. Kammlade, Jr. 1955. Diseases and parasites. Page 477 in Sheep Science Revised Edition. J. B. Lippincott Company, Chicago, IL, Philadelphia, PA, and New York, NY.

Kaplan, R. M., J. M. Burke, T. H. Terrill, J. E. Miller, W. R. Getz, S. Mobini, E. Valencia, M. J. Williams, L. H. Williamson, M. Larsen, and A. F. Vatta. 2004. Validation of the FAMACHA eye color chart for detecting clinical anemia in sheep and goats on farms in the southern United States. Vet. Parasitol. 123:105-120.

Keen, C. L. and T. W. Graham. 1989. Trace elements. Pages 757-765 in Clinical Biochemistry of Domestic Animals. 4th ed. J. J. Kaneko ed. Academic Press, San Diego, CA.

Kline, R. D., V. W. Hays, and G. L. Cromwell. 1971. Effects of copper, molybdenum and sulfate on performance, hematology and copper stores of pigs and lambs. J. Anim. Sci. 33:771-779.

Knox, M. R., J. W. Steel, C. A. Anderson, and L. L. Muir, ed. 2003. Nutrition-parasite interactions in sheep. Aust. J. Exp. Agric. (Special Issue). 43:1383-1488.

Kramer, J. W. 1989. Clinical Enzymology. Pages 354-356 in Clinical Biochemistry of Domestic Animals. 4th ed. J. J. Kaneko ed. Academic Press, San Diego, CA.

Leathwick, D. M., A. Vlassoff, and N. D. Barlow. 1995. A model for nematodiasis in New Zealand lambs: the influence of drenching and grazing management on the development of anthelmintic resistance. Int. J. Parasitol. 25:1479-1491.

Leathwick, D. M., C. M. Miller, A. E. Brown, and I. A. Sutherland. 1999. The establishment rate of *Ostertagia circumcincta* and *Trichostrongylus colubriformis* in lactating Romney ewes. Int. J. Parasitol. 29:315-320.

Linder, M. C, and M. Hazegh-Azam. 1996. Copper biochemistry and molecular biology. Am. J. Clin. Nutr. 63:797S-811S.

Linder, M. C., L. Wooten, P. Cerveza, S. Cotton, R. Shulze, and N. Lomeli. 1998. Copper transport. Am. J. Clin. Nutr. 67(suppl): 965S- 971S.

Malan, F. S., J. A. Van Wyk, and C. D. Wessels. 2001. Clinical evaluation of anaemia in sheep: early trials. Onderstepoort J. Vet. Res. 68:165-174.

Malone, J. B. 1983. Research needs and priorities for ruminant internal parasites in the United States. Am. J. Vet. Res. 44:1836-1847.

Martin, P. J., N. Anderson, and R. G., Jarrett. 1989. Detecting benzimidazole resistance with faecal egg count reduction tests and in vitro assays. Aust. Vet. J. 66:236-240.

McAnulty, R. M., A. S. Familton, R. D. Sedcole, and A. R. Sykes. 2001. Changes in the resistance of the ewe to infection with *Teladorsagia circumcincta* during late pregnancy and lactation. Anim. Sci. 72:159-168.

Michel, J. F. 1976. The epidemiology and control of some nematode infections in grazing animals. Adv. Parasitol. 146:66-76.

Miller, J. E., and D. W. Horohov. 2006. Immunological aspects of nematode parasite control in sheep. J. Anim. Sci. 84(E. Suppl.):E124-132.

Miller, J. K., N. Ramsey, and F. C. Madsen. 1988. The trace elements. Page 347-357 in The Ruminant Animal: Digestive Physiology and Nutrition. D. C. Church, ed. Waveland Press Inc., Illinois.

Milne, D. B. and P. H. Weswig. 1968. Effect of supplementary copper on blood and liver copper-containing fractions in rats. J. Nutr. 95:429-433.

Min, B. R. and S. P. Hart. 2003. Tannins for suppression of internal parasites. J. Anim. Sci. 81(E. Suppl. 2):E102-E109.

Molento, M. B., A. A. Gavião, R. A. Depner, and C. C. Pires. 2009. Frequency of treatment and production performance using the FAMACHA method compared with preventive control in ewes. Vet. Parasitol. 162:314-319.

Niezen, J. H., W. A. G. Charleston, J. Hodgson, A. D. Mackay, and D. M. Leathwick. 1996. Controlling internal parasites in grazing ruminants without recourse to anthelmintics: approaches, experiences and prospects. Int. J. Parasitol. 26:983-992.

Niezen, J. H., H. A. Robertson, G. C. Waghorn, W. A. G. Charleston. 1998. Production, faecal egg counts and worm burdens of ewe lambs which grazed six contrasting forages. Vet. Parasitol. 80:15-27.

Nighbert, E. M. 1932. Controlling stomach worms in sheep and lambs. Leaflet No. 89. U.S. Dept. Agric., Washington, DC.

NRC. 1984. Pages 48-61 in Nutrient Requirements of Beef Cattle. 6th rev. ed. Natl. Acad. Press, Washington, DC.

NRC. 2005. Pages 134-153 in Mineral Tolerance of Domestic Animals. 2nd rev. ed. Natl. Acad. Press, Washington, DC.

O'Connor, L. J., S. W. Walkden-Brown, and L. P. Kahn. 2006. Ecology of the free-living stages of major trichostrongylid parasites of sheep. Vet. Parasitol. 142:1-15.

O'Dell, B. L. 1981. Roles for iron and copper in connective tissue biosynthesis. Phil. Trans. R. Soc. Lond. B 294:91-104.

Papadopoulos, E. 2008. Anthelmintic resistance in sheep nematodes. Small Rumin. Res. 76:99-103.

Pomroy, W. E. 2006. Anthelmintic resistance in New Zealand: a perspective on recent findings and options for the future. New Zealand Vet. J. 54:265-270.

Pond, W. G., D. C. Church, and K. R. Pond. 1995. Inorganic mineral elements. Pages 199-203 in Basic Animal Nutrition and Feeding. 4th ed. John Wiley and Sons, New York, NY.

Poppi, D. P., J. C. MacRae, A. Brewer, and R. L. Coop. 1986. Nitrogen transactions in the digestive tract of lambs exposed to the intestinal parasite *Trichostrongylus colubriformis*. Brit. J. Nutr. 55:593-602.

Reed, H. E. 1930. A study in the control of stomach worms. J. Anim. Prod. 1930: 109-111.

Rietz, J. H. 1935. Copper sulfate as an anthelmintic for gastro-intestinal parasites of sheep. Bull. No. 264. Agric. Exp. Sta. West Virginia Univ., Morgantown.

Ross, D. B. 1966. The diagnosis, prevention and treatment of chronic copper poisoning in housed lambs. Brit. Vet. J. 122:279-284.

Sangster, N. C. 1999. Anthelmintic resistance: past, present and future. Int. J. Parasitol. 29:115-124.

Saylor, W. W. and R. M. Leach Jr., 1980. Intracellular distribution on copper and zinc in sheep: effect of age and dietary levels of the metals. J. Nutr. 110:448-459.

SID. 2002. Health. In: Sheep Production Handbook. Page 473,711. Am. Sheep Ind. Assoc., Inc. C & M Press, Denver, CO.

Shorb, D. A. 1944. Factors influencing embryonation and survival of eggs of the stomach worm *Haemonchus contortus*. J. Agric. Res. 69:279-287.

Smith, A. F. 1972. Separation of tissue and serum creatine kinase isoenzymes on polyacrylamide gel slabs. Clin. Chem. Acta. 39:351-359.

Steel, J. W., W. O. Jones, and L. E. A. Symons. 1982. Effects of a concurrent infection of *Trichostrongylus colubriformis* on the productivity and physiological and metabolic responses of lambs infected with *Ostertagia circumcincta*. Aust. J. Agr. Res. 33:131-140.

Stromberg, B. E. 1997. Environmental factors influencing transmission. Vet. Parasitol. 72: 247-264.

Suttle, N. F. 1983. Effects of molybdenum concentration in fresh herbage, hay and semi-purified diets on the copper metabolism of sheep. J. Agric. Sci., Camb. 100:651-656.

Symons, L. E. A. 1985. Anorexia: occurrence, pathophysiology and possible causes in parasitic infection. Adv. Parasit. 24:103-133.

Szaua, G., W. Gruber, and E. Bernt. 1976. Creatine kinase in serum: 1. Determination of optimum reaction conditions. Clin. Chem. 22:650-656.

Taylor, M. A., K. R. Hunt, and K. L. Goodyear. 2002. Anthelmintic resistance detection methods. Vet. Parasitol. 103:183-194.

Thorp, W. T. S., W. L. Henning, and J. F. Shigley. 1945. Phenothiazine as an anthelmintic for breeding ewes. J. Anim. Sci. 4:133-140.

Todd, A. C., G. W. Kelley, and M. F. Hansen. 1949. Winter survival of sheep parasites on a pasture in Kentucky. Bulletin No. 533. Univ. of Kentucky, Lexington.

Todd, J. R. 1969. Chronic copper toxicity of ruminants. Proc. Nutr. Soc. 28:189-198.

Todd, J. R. and R. H. Thompson. 1963. Studies on chronic copper poisoning: II. Biochemical studies on the blood of sheep during the haemolytic crisis. Brit. Vet. J. 119:161-173.

Torres-Acosta, J. F. J and H. Hoste. 2008. Alternative or improved methods to limit gastro-intestinal parasitism in grazing sheep and goats. Small Rumin. Res. 77: 159-173.

Turner, J. C., V. Shanks, P. J. Osborn, and S. M. Gower. 1987. Copper absorption in sheep. Comp. Biochem. Physiol. 86C:147-150.

Underwood, E. J. and N. F. Suttle. 1999. Pages 283-342 in The Mineral Nutrition of Livestock. 3rd ed. CABI Publishing, New York, NY.

Vanimisetti, H. B., S. L. Andrews, A. M. Zajac, and D. R. Notter. 2004. Inheritance of fecal egg count and packed cell volume and their relationship with production traits in sheep infected with *Haemonchus contortus.* J. Anim. Sci. 82:1602-1611.

Van Wyk, J. A. 2001. Refugia: overlooked as perhaps the most potent factor concerning the development of anthelmintics resistance. Onderstepoort J. Vet. Res. 68:55-67.

Van Wyk, J. A. and G. F. Bath. 2002. The FAMACHA system for managing haemonchosis in sheep and goats by clinically identifying individual animals for treatment. Vet. Res. 33:509-529.

Vercruysse, J. 2005. Anthelmintics. Page 2121 in The Merck Veterinary Manual 9th ed. C. M. Kahn, ed. Merck & Co. Inc., Whitehouse Station, NJ.

Vlassoff, A., D. M. Leathwick, and A. C. G. Heath. 2001. The epidemiology of nematode infections of sheep. New Zealand. Vet. J. 49:213-221.

Waller, P. J. 1993. Control strategies to prevent resistance. Vet. Parasitol. 46:133-142.

Waller, P. J. 1997. Anthelmintic resistance. Vet. Parasitol. 72:391-412.

Waller, P. J. 1999. International approaches to the concept of integrated control of nematode parasites of livestock. Int. J. Parasitol. 29:155-164.

Weiss, K. C. and M. C. Linder. 1985. Copper transport in rats involving a new plasma protein. Am. J. Physiol. 249:E77-88.

Whitlock, H. V. 1948. Some modifications of the McMaster helminth egg-counting technique and apparatus. J. Counc. Sci. Ind. Res. 21:177-180.

Williams, J. C. 1997. Anthelmintic treatment strategies: current status and future. Vet. Parasitol. 72:461-477.

Woolaston, R. R. 1992. Selection of Merino sheep for increased and decreased resistance to *Haemonchus contortus*: peri-parturient effects on faecal egg counts. Int. J. Parasitol. 22:947-953.

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Publications:

Aaron, D. K., D. G. Ely, J. Wyles, R. A. Zinner, A. K. Lunsford, and M. L. Mallory. 2006. A nutritional supplement for beef cattle grazing endophyte-infected tall fescue. II. Response of individually-pastured cow/calf pairs. J. Anim. Sci. 84(Suppl. 1):396.

Aaron, D. K., D. G. Ely, E. Fink, B. T. Burden, and M. M. Simpson. 2008. Carcass composition of Polypay verses White Dorper x Polypay crossbred lambs. J. Anim. Sci. 86(Suppl. 3):114.

Aaron, D. K., D. G. Ely, E. Fink, B. T. Burden, M. M. Simpson, and A. K. Lunsford. 2008. Phenotypic correlations among growth and carcass traits of Polypay and White Dorper crossbred lambs. J. Anim. Sci. 86 (Suppl. 2):340.

Aaron, D. K., D. G. Ely, E. Fink, B. T. Burden, and M. M. Simpson. 2009. Factors affecting indicators of *Haemonchus contortus* infection in Polypay and percentage White Dorper ewes. J. Anim. Sci. 87 (E Suppl. 3):108. (Abstr.)

Aaron, D. K., M. M. Simpson, D. G. Ely, E. Fink, B. T. Burden, M. E. Hoar, J. Farris. 2010. Accuracy of FAMACHA system for estimating degree of

Haemonchus contortus induced anemia in Polypay and percentage White Dorper ewes. J. Anim. Sci. (Accepted).

Ely, D. G., D. K. Aaron, J. Wyles, R. A. Zinner, A. K. Lunsford, and M. L. Mallory. 2006. A nutritional supplement for beef cattle grazing endophyte-infected tall fescue. I. Response of herd-managed cows and calves. J. Anim. Sci. 84(Suppl. 1):396.

Lunsford, A. K., D. G. Ely, D. K. Aaron, M. L. Mallory, and R. A. Zinner. 2006. Nutrient utilization in Polypay and percentage White Dorper lambs fed a high roughage and high concentrate diet. J. Anim. Sci. 84(Suppl. 2):2.

Lunsford, A. K., D.G. Ely, D. K. Aaron, M. M. Simpson, and R. A. Zinner. 2008. Nutrient utilization in Polypay and percentage White Dorper lambs fed a high roughage and high concentrate diet. Sheep & Goat Res. J. 28:1-6.

Lunsford, A. K., S. F. Liao, D. K. Aaron, M. M. Simpson, D. G. Ely, J. A. Boling, and J. C. Matthews. 2010. Content of liver, kidney, longissimus muscle, and subcutaneous fat ALT was lower in growing White Dorper crossbred lambs and was coincidental with more heavily muscled carcasses. J. Anim. Sci. (Submitted).

Simpson, M. M., D. K. Aaron, E. Fink, B. T. Burden, and D. G. Ely. 2008. Control of *Haemonchus contortus* infestation in ewes with copper sulfate. J. Anim. Sci. 86(Suppl. 3):127.

Zinner, R. A., D. G. Ely, D. K. Aaron, L. P. Bush, A. K. Lunsford, and M. L. Mallory. 2006. Adaption of lambs to an endophyte-infected tall fescue seed diet. J. Anim. Sci. 84(Suppl. 2):5.

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